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TRANSLOCATION OF MINERAL CONSTITUENTS OF SEEDS AND TUBERS OF CERTAIN PLANTS DURING GROWTH

By G. DAVIS BUCKNER,[1]
Chemist, Kentucky Agricultural Experiment Station

INTRODUCTION

Several years ago it was observed by Dr. J. H. Kastle, Director of the Kentucky Experiment Station, that the morning-glory vine (*Ipomoea purpurea*) after removal from the soil would continue to grow when its roots were immersed in rain water. Often the growth of this vine attained a length of several feet, bloomed, and produced seeds. During this period the lower leaves etiolated, withered, and ultimately dried up. Evidently the new growth attained by this plant under these conditions was largely at the expense of the various materials contained in the roots, the lower part of the stem, and the lower leaves; especially was this true of the mineral matter required by the new growth, inasmuch as no mineral substance was supplied by the rain water. It therefore occurred to Dr. Kastle that it would be of interest to determine the translocation of the mineral matter in this vine under these conditions. Accordingly, a number of morning-glory vines were completely removed from the soil in which they had grown, and the soil was carefully washed from their roots, which were placed in wide-mouth bottles containing distilled water, the vines being trained on strings arranged vertically in a window. Under these circumstances the vines were found to increase in length by several feet. They put out new roots and a large number of new leaves and in many instances bloomed and produced seeds. Unfortunately, with the limited space at our disposal we were unable to secure a sufficient amount of material to determine the translocation of the mineral substances of the plants under these conditions, and it was found necessary to abandon the experiment with the morning-glory for the time being. However,

[1] The writer wishes to acknowledge the many valuable suggestions made by Dr. Kastle during the progress of these experiments.

we are still of the opinion that on account of its hardiness under all sorts of conditions this plant would lend itself better than any other to such studies as those herein contemplated, and we hope to take it up again at some future time.

In thinking over the subject of the translocation of mineral matter during plant growth it occurred to us that it might be of interest to determine the translocation of the mineral matter contained in the seeds and tubers of certain plants during the period of sprouting. Therefore, our present experiments have been confined to the seeds of the garden bean (*Phaseolus vulgaris*), corn (*Zea mays*), and to the potato tuber (*Solanum tuberosum*). Up to this time our work has been confined to the measurement of the translocation of phosphorus, calcium, potassium, magnesium, and silicon.

EXPERIMENTS WITH GARDEN BEANS

The cotyledons of the garden bean were found to contain a considerable amount of mineral matter, and the seedlings of this plant are hardy and well adapted to our requirements. The only difficulties experienced in growing these seedlings under the conditions of these experiments were the growth of molds and the attack of the seedling by the damping-off wilt. The bean in this instance was germinated and allowed to grow to maturity at the expense of the food stored in the cotyledons, extreme care being taken that they should receive no mineral food from external sources. We, of course, realized that the growth of any plant in distilled water is more or less abnormal; yet these beans germinated and produced perfect seedlings with well-developed leaves.

Great difficulty was experienced in keeping down the growth of molds during the process of germination and in preventing the damping-off wilt from attacking the seedlings. In order to overcome these difficulties, every precaution was taken to sprout and grow these seedlings under aseptic conditions. The distilled water employed was boiled for 20 minutes before coming in contact with the beans. The germination and growth of the seedlings were carried out in a dust-proof closet constructed for that purpose. A framework of wood was made and covered inside and out with cheesecloth, leaving an air space of about 2 inches. During the experiment both layers of the cheesecloth were kept moistened with a 50 per cent solution of glycerin and water. This prevented dust and spores from entering the closet; yet it allowed a free passage of air and light. An opening was made in the side of the closet just large enough to admit the head and shoulders of a man. Over this opening was hung a curtain, so arranged as to exclude dust while working inside and when the closet was closed.

The seedlings were never allowed to come in contact with glass. The germinations were made in large porcelain evaporating dishes in which

were placed round perforated porcelain plates, similar to those used in desiccators, on top of which were placed two circular pieces of blotting paper which had been treated with dilute hydrochloric acid and washed free from chlorids with distilled water. Small lamp wicks connected these blotters with the water in the bottom of the dish, so that they would remain moist during the period of germination. Just previous to placing the beans between the blotters the entire apparatus was sterilized by heating at 180° C. for two hours.

The germinated beans were transplanted to test tubes which had been carefully paraffined inside and in each of which was placed a plug of cotton about half an inch from the top and held in place by a small amount of paraffin. The cotton was the purest we could obtain and was treated with dilute hydrochloric acid and washed with sterile distilled water until no test for chlorids could be obtained. This cotton gave practically no ash when incinerated.

In beginning this experiment 1,400 perfect beans were selected, cleaned with a damp cloth, and divided into two lots of 700 each. These lots were labeled "A" and "B," respectively. The 700 beans labeled "A" were placed in a flask and covered with 95 per cent alcohol containing 20 per cent of formalin and allowed to stand for 20 minutes. The beans were then drained and washed free from alcohol with sterile distilled water. The alcoholic drainage and washings were evaporated to dryness and saved for analysis, being labeled "11" in Table I. The beans were now transferred to the sterile germinating dishes described above and placed between blotters, care being taken that the beans did not touch each other. Throughout the germination of the beans sterile distilled water was added in just sufficient amounts to keep the beans moist. Germination started at once, and the small radicle appeared in from two to three days and in some instances was half an inch in length by the end of the fourth day. As soon as this stage was reached, the integuments were removed from the cotyledons with sterile, platinum-tipped forceps, care being taken not to bruise the cotyledons nor allow dust or dirt to come in contact with them. The integuments were preserved and labeled "9" in Table I. The seedlings were then transferred to paraffined test tubes ¾ by 6 inches, the seedlings being held in place with a small quantity of sterile cotton. The test tubes were filled with sterile distilled water, which was replaced as fast as it was removed by the plant or by evaporation. The seedlings began to grow immediately, putting forth roots and plumules. Some of the beans on germinating proved to have imperfect cotyledons; these with a number which had been bruised during the removal of the integuments were discarded, so that at the end of the experiment only 609 seedlings had been allowed to mature. This number furnished the material for analysis.

TABLE I.—*Analysis of separate parts of bean seedlings and whole beans*

(A) SEEDLINGS

Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorus as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.	
		Gm.	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*
1,218 Cotyledons (exhausted)	7	70.8239	3.3441	4.72	1.1768	35.18	0.0819	2.45	0.0959	2.87	1.5459	46.52	0.0367	1.10
609 Integuments (removed)	9	16.5423	.6372	3.85	.0121	1.91	.1965	30.85	.0469	7.37	.1224	19.22	.0137	2.00
609 Roots	8	7.5516	.7785	10.31	.1916	24.61	.0206	2.65	.0129	1.67	.2993	38.46	.0175	2.25
609 Upper stems	6	7.1577	.5611	7.83	.1441	25.69	.0060	1.07	.0097	1.74	.2084	37.16	.0062	1.10
609 Lower stems	5	21.6122	1.1339	5.24	.3740	32.99	.0278	2.45	.0341	3.01	.4224	37.26	.0085	.75
1,218 Leaves	4	31.1819	2.1048	6.78	.6064	29.00	.0157	.95	.0574	2.73	.9539	45.32	.0210	1.00
Drain	11	3.9526	1.2335	31.20	.2026	18.12	.0242	1.96	.0628	5.09	.5996	48.61	.0102	.82
Total weight	.....	158.8222	9.7931	.....	2.7076	.....	.3727	.....	.3197	.....	4.1519	.....	.1138	.....

(B) CONTROL BEANS

1,218 Whole cotyledons	3	169.3022	7.3058	4.31	2.5000	34.22	0.0949	1.30	0.2388	3.27	3.7038	49.19	0.0403	0.55
609 Integuments	2	16.4422	.7369	4.48	.0251	3.41	.2505	34.00	.0424	5.76	.1786	24.24	.0081	1.10
Drain	1	2.7616	.6354	23.07	.0582	9.16	.0176	2.77	.0237	3.74	.2823	44.44	.0117	1.85
Total weight	.....	188.5060	8.6781	.....	2.5833	.....	.3620	.....	.3049	.....	4.1647	.....	.0621	.....

As the growth of the seedlings proceeded, the cotyledons began to shrink and finally turned brown. The root development in all cases was good, nearly filling the test tubes, and each seedling developed two perfect leaves. The seedlings were allowed to grow until they began to etiolate and wilt, this period being reached in from 17 to 22 days. The plants thus grown were very uniform in size and development, the average height being 6½ inches. During their development care was taken that they should not touch each other. As fast as they matured, they were removed from the test tubes and the cotton carefully removed from the stem and roots. The plants were then divided into roots (8),[1] lower stems (5) which averaged 4½ inches in height, exhausted cotyledons (7), upper stems (6) which averaged 2 inches, and the leaves (4). The liquid remaining in the test tubes was evaporated to dryness and added to the washings (11).

Six hundred and nine selected beans labeled "B" received the same treatment as those labeled "A," except they were allowed to live only until the radicle had appeared and the integument had softened. The integument (2) and the cotyledons (3) were carefully air-dried, as were the above-mentioned plants. The drainage and washings (1) from these beans were carefully evaporated to dryness. These several parts of the beans were analyzed to check the analyses of the seedlings, the results of which are given in Table I.

In analyzing the separate portions of the air-dried material which had been carefully ashed at a dull-red heat, three portions of 0.2000 gm. each were carefully weighed out. In one portion phosphorus and silica were determined, while in another portion the determination of potassium was made. The methods used were essentially the official methods of the Association of Official Agricultural Chemists.[2] In a third portion of the ash, calcium and magnesium were determined according to the method of McCrudden.[3]

In Table I are to be found the results of the analyses of the separate portions of 609 seedlings and the separate parts of 609 beans.

It is evident from the results given in Table I that the weight of the total ash of the seedlings agrees fairly well with the total weight of the ash of the bean control, the difference being due in all probability to unavoidable outside contamination during the period of growth. The comparative analyses of the inorganic constituents fall well within the limit of experimental error. The greatest difference is observed in the case of silica, the seedlings containing nearly twice as much as the beans.

[1] The numbers in parentheses refer to the number of part in the tables.

[2] Wiley, H. W., et al. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908.

[3] McCrudden, F. H. The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine, and feces. *In* Jour. Biol. Chem., v. 7, no. 2, p. 83–100. 1910.

—— The determination of calcium in the presence of magnesium and phosphates; the determination of calcium in urine. *In* Jour. Biol. Chem., v. 10, no. 3, p. 187–199. 1911.

This is probably due to unavoidable contamination. It is of interest to note that the integument contains 52.72 per cent of the total calcium oxid found in the bean; it is also interesting to find that the amount of phosphorus and potassium in the integument is very small. It is shown that a marked accumulation of the mineral elements in the leaves and lower stems occurs during growth. This is more clearly shown where the results are expressed as the percentage distribution of the mineral constituents that actually migrated from the cotyledons, as seen in Table II.

TABLE II.—*Percentage distribution of the mineral constituents of bean seedlings*

Part.	Part No.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2)
Cotyledons (exhausted)	1	47.20	54.53	45.67	45.07	40.82
Roots	4	7.68	13.72	6.14	8.72	19.47
Upper stems	5	5.78	3.99	4.62	6.07	6.90
Lower stems	3	15.00	18.51	16.24	12.31	9.45
Leaves	2	24.34	10.45	27.33	27.83	23.46

In the foregoing experiment we have germinated beans, and they have grown until they died from the want of nourishment. From all physical appearances the growth of the seedlings has been normal. This growth has been at the expense of the food material stored in the cotyledons, the carbon dioxid inspired from the air, and the distilled water received through the roots. Every precaution was taken to exclude all mineral matter from external sources. Referring to Table II, it is seen that approximately 50 per cent of the total mineral content of the cotyledons remained unused and that approximately 50 per cent was translocated to different parts of the seedlings during growth. As might be expected, the greatest quantity of these elements migrate to the leaves and the next greatest quantity locate in the lower stems. The large amount of calcium and silica locating in the roots is also of interest.

These results serve to emphasize the importance of the mineral matter both to the seedlings and to the sprouting seed or cotyledon. In other words, it would seem from these results that the mineral matter originally present in the seed or in the cotyledons functions in the act of sprouting in two different ways: First, to promote the enzymic changes occurring in the sprouting cotyledons and seeds themselves; and, in the second place, to support the growth and development of the seedlings. The growth will therefore depend somewhat at least on the total mineral matter originally present in the cotyledons or seeds, a part of this being translocated to meet the requirements of the growing seedling. Approximately an equal part or, at any rate, a relatively large amount of the mineral matter remains in the seed or cotyledon to support and promote those enzymic changes characteristic of the seed or cotyledon in an active katabolic condition.

EXPERIMENTS WITH CORN

Similar experiments have been tried with corn, except that the seedlings were grown in aluminum cups instead of in paraffined tubes. One thousand grains of corn were germinated, transferred to aluminum cups, and allowed to grow for 23 days, when they began to etiolate. During this time these seedlings attained a height of 9 inches. At this point they were removed from the cups and dissected as follows: Leaves (2), exhausted cotyledons (3), stems (4), and roots (5). (See Table III.) These were controlled by the same number of whole corn grains (1) as given also in Table III. These several lots of material were analyzed in the same manner as the bean seedlings. In this experiment we have also followed the translocation of iron and aluminum. Unfortunately, the results obtained with these two last-named elements show contamination from the aluminum cups used in the experiment. The results of the analyses of the ash of corn grain and of the several parts of the seedlings thereof are given in Table III.

It will be seen from the results of these analyses that the sum of the total ash of the several parts of the corn seedling exceeds the total ash of the corn grain by 0.9487 gm. This is doubtless to be explained by the fact that iron and aluminum were taken up in considerable amounts from the cups and also by contamination with small amounts of dust from the outside air. It will be seen that the sum of the amounts of phosphoric acid, potash, and magnesia in the several parts of the corn seedling agrees with that of the corresponding amounts of these substances found in the corn grain, within the limits of experimental error. A point of interest in this connection is that magnesia is greatly in excess of lime in the grain of corn and in the several parts of the seedling obtained therefrom. The amounts of lime, silicon, iron, or aluminum found in the several parts of the seedling are in excess of the amounts of these substances found in the grain. As already pointed out, this discrepancy is doubtless due to outside contamination. Under the conditions prevailing in this experiment approximately two-thirds of the total mineral matter of the corn grain has been translocated to the stems, roots, and leaves of the seedling during the process of growth. It is evident further that approximately the same amounts of this mineral matter go to stem and roots, respectively, whereas a somewhat larger amount of the mineral matter migrates to the leaves of the seedlings. The fact that a relatively large amount of the mineral matter, amounting in this case to something over one-third of the whole, remains in the exhausted cotyledon is of interest and doubtless has the same significance for the growth of the seedling as is believed to obtain in the case of the bean, already discussed. The percentage distribution of the mineral constituents of corn during the growth of the seedling is shown in Table IV.

TABLE III.—*Analysis of separate parts of corn seedlings and whole corn grains*

SEEDLINGS

Part.	No. of part.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried material.	Phosphorous as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.		Iron oxid (Fe_2O_3) in ash.		Aluminum oxid (Al_2O_3) in ash.	
		Gm.	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*
3,000 Leaves	2	43.2716	2.0107	4.64	0.7845	39.02	0.0502	2.50	0.1425	7.09	0.6428	31.92	0.0613	3.05	0.0012	0.06	0.0096	0.48
1,000 Cotyledons (exhausted)	3	342.3911	2.7254	.79	2.2858	47.18	.0735	2.70	.2335	8.57	.3597	13.20	.1812	6.65	.0234	.86	.0136	.50
1,000 Stems	4	36.7137	1.3292	3.62	.5186	39.02	.0221	1.65	.0835	6.29	.3808	28.65	.0198	1.50	.0107	.81	.0086	.65
1,000 Roots	5	48.9321	1.3514	2.76	.3849	28.46	.0216	1.60	.0369	2.73	.4153	29.92	.0406	3.45	.0120	.89	.2471	18.29
Total	.....	471.3085	7.4167	.....	2.9738	.....	.1674	.....	.4964	.....	1.7986	.....	.3089	.....	.0473	.....	.2789	.....

WHOLE GRAINS

1,000 Whole corn	1	461.9412	6.4680	1.40	3.0250	46.77	0.0562	0.87	0.4760	7.36	1.8929	29.25	0.0822	1.28	0.0116	0.18	0.0051	0.079

TABLE IV.—*Percentage distribution of the mineral constituents of corn seedlings*

Part.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2).	Iron oxid (Fe_2O_3).	Aluminum oxid (Al_2O_3).
Leaves	26. 38	30. 02	28. 71	35. 74	19. 84	2. 54	3. 44
Exhausted cotyledons	43. 24	43. 86	47. 04	20. 00	58. 66	49. 47	4. 88
Stems	17. 44	13. 21	16. 82	21. 17	6. 41	22. 62	3. 08
Roots	12. 94	12. 91	7. 43	23. 09	15. 09	25. 37	88. 60

A comparison of these individual mineral constituents shows that except in the case of potash approximately 50 per cent thereof have been translocated from the cotyledons to the several parts of the seedling during growth, and that the translocation of potash is greatly in excess of that of the other mineral constituents. The taking up of such considerable amounts of iron and aluminum from the aluminum cups in which these seedlings had been grown and the great accumulation of aluminum on the roots of the seedlings is also a matter of interest, although it has no immediate bearing on the subject under consideration. It is also evident from the foregoing that there is a decided accumulation of translocated mineral matter in the leaves of the seedling, a fact that is in harmony with the rapid growth of the leaves as compared with that of the other parts of the seedling.

EXPERIMENTS WITH POTATOES

In the experiment with the potato tuber we have allowed potatoes to sprout in a dark closet, after they had been thoroughly cleaned. When the tubers began to soften they were removed from the dark closet and the sprouts (1) cut off. The potato was then carefully pared and the skin (2) and the starchy tissue (3) were carefully dried and ashed and the quantities of calcium, magnesium, phosphorus, potassium, and silicon were determined. The results are shown in Table V.

TABLE V.—*Analysis of separate parts of sprouted potatoes*

Part.	Part No.	Total weight of air-dried material.	Total weight of ash.	Ash in air-dried solids.	Phosphorus as P_2O_5 in ash.		Calcium oxid (CaO) in ash.		Magnesium oxid (MgO) in ash.		Potassium as K_2O in ash.		Silica (SiO_2) in ash.	
		Gm.	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*	*Gm.*	*Per ct.*
New sprouts	1	27. 3722	2. 1185	9. 91	0. 2661	12. 56	0. 0190	0. 90	0. 0443	2. 72	0. 8558	40. 40	0. 0201	0. 95
Skin	2	46. 9359	3. 8205	8. 14	. 2250	5. 89	. 0649	1. 70	. 0518	1. 33	1. 5408	40. 33	. 3228	8. 45
Tubers (exhausted)	3	185. 3766	8. 1120	4. 37	1. 0058	12. 40	. 0608	. 75	. 1835	2. 25	4. 3415	53. 52	. 0486	. 60
Total weight		253. 6847	14. 0510		1. 4969		. 1447		. 2796		6. 7381		. 3915	

The relatively high percentage of ash in the sprout of the potato as compared with that contained in the exhausted tuber is a matter of interest. It will be seen, however, that considerable amounts of ash still remain in the exhausted tuber after the growth of the sprouts is complete, indicating the necessity of mineral matter for those changes occurring in the tuber during the act of sprouting. Table VI gives the percentage distribution of the several mineral constituents between the sprouts and exhausted tubers, including the skin.

TABLE VI.—*Percentage distribution of the mineral constituents of potatoes*

Part.	Phosphorus as P_2O_5.	Calcium oxid (CaO).	Magnesium oxid (MgO).	Potassium as K_2O.	Silica (SiO_2).
Sprouts	17.77	13.12	15.84	12.68	5.13
Tubers (exhausted)	67.13	42.02	65.68	64.43	12.41

In Table VI it is observed that a large amount of the mineral material remains unused in the exhausted tuber of the potato and that approximately only 15 per cent of the different mineral constituents have migrated to the sprouts.

CONCLUSIONS

The most striking fact brought out thus far by these studies on the translocation of the mineral matter of the seed and tuber during the growth of the seedling is the retention of considerable amounts of the mineral matter, varying from 46.66 per cent in the garden bean and 38.66 per cent in corn to 50.33 per cent in the potato tuber in the cotyledons and tuber, respectively. As indicated in the foregoing experiments, this probably finds its explanation in the necessity for definite amounts of the various mineral constituents to promote the katabolic changes occurring in the cotyledon and tuber during sprouting. So far as could be ascertained, there were no very striking differences in the quantities of its several mineral constituents translocated and no marked selective influences shown by the roots, stem, and leaves of the growing seedling for any particular mineral reserve material contained in the seed or tuber. Up to the present time, great difficulty has been experienced in the selection of a suitable container in which to grow these seedlings. This has proved a serious obstacle to this work. It is hoped, however, that this difficulty may be finally overcome and better and more constant results obtained through the use of pure paraffin containers.

FATE AND EFFECT OF ARSENIC APPLIED AS A SPRAY FOR WEEDS

By W. T. McGeorge,
Chemist, Hawaii Agricultural Experiment Station

INTRODUCTION

In certain districts of Hawaii during the rainy season cultivation is impracticable, because of its bad effect upon the texture of the soil. Yet at times this season is abnormally long and especially favorable to the growth of weeds. Weed control is therefore a very important problem for Hawaiian planters. In experiments at the Hawaii Experiment Station [1] it was found that the most economical means of weed control under such conditions lay in the use of chemical sprays. Careful comparative tests were made of such chemicals as sodium arsenite, ferrous sulphate, carbon bisulphid, etc. Of these, sodium arsenite proved by far the most effective and was recommended for use. Sodium arsenite sprays have now been used in Hawaii for weed eradication for about five years and have proved to be efficient and economical. Such sprays have not only been used to replace hand labor in the fields, but also as a means of ridding grass lands of undesirable plants.

In view of the possible injury to soils and crops as a result of the continued use of such sprays, the Hawaii Experiment Station undertook a study of the fate in the soil of the arsenic so applied and its influence upon plant growth and upon ammonification and nitrification.

EFFECT OF SODIUM ARSENITE ON PLANT GROWTH

Apparently there is little or no immediate danger to crops from the use of sodium arsenite as a spray. In fact, in experiments with millet, buckwheat, and cowpeas grown on three different types of Hawaiian soils it was found that small quantities of arsenic stimulate plant growth. However, analyses of the plants did show that the arsenic is assimilated and that when it is present in the tissues in sufficient concentration death of the plant results.

The most surprising feature of the investigation was the influence on the ammonifying and nitrifying bacteria. In one type of soil ammonification was stimulated even by such excessive amounts as 1 per cent of arsenic (As_2O_3) in the soil. The results as a whole indicate that no fear need be entertained regarding any detrimental influences toward the

[1] Wilcox, E. V. Killing weeds with arsenite of soda. Hawaii Agr. Exp. Sta. Press Bul. 30, 15 p. [1911.]
Krauss, F. G. Suppression of weeds among pineapples by arsenite of soda spray. Hawaii Agr. Exp. Sta. Press Bul. 48, 8 p., 2 fig. 1915.
McGeorge, W. T. The effect of arsenite of soda on the soil. Hawaii Agr. Exp. Sta. Press Bul. 50. 16 p., 3 fig. 1915.

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organisms upon which the plants rely for their available nitrogen, provided proper soil texture is maintained.

Furthermore, it was found that in time the arsenic practically loses its toxic influence toward plants. This was shown by the comparative growth of plants on soils treated at time of seeding and those seeded several months following the application of the arsenic to the soil. There are only two possible explanations of this condition: Either the arsenic reacts with certain of the soil constituents, resulting in a less toxic combination, or it is rapidly leached from the soil.

ABSORPTION OF ARSENIC BY THE SOIL

When a soluble salt is added to a soil, its ultimate disposition must depend upon certain chemical reactions and physical phenomena. In this case the possibilities involve (1) a combination with or replacement of salts already present, resulting in its absorption as a whole; or (2) a selective absorption involving the fixation of only one ion of the salt.

In order to determine the fate of arsenic and the effect of irrigation, a set of lysimeter experiments was inaugurated.

LYSIMETER EXPERIMENTS

Three types of soil were selected: (1) A ferruginous red clay, (2) a ferruginous brown clay, and (3) a highly organic silt. Twenty-five pounds of soil were placed in each of six lysimeters, two being filled with each type. To each soil were added 3 liters of a solution of sodium arsenite of the same strength as that used for spraying weeds. One series of three was allowed to stand for two months protected from rain. To the other three 1 liter of water was added every other day for several weeks, after which the soil was allowed to stand in the lysimeter until dry enough to sample.

The object of these experiments was to determine the rate of fixation, the depth to which the arsenic can penetrate, and the leaching effect of irrigation. At the expiration of the above time samples were taken at various depths in the lysimeters and the percentage of arsenic (As_2O_3) in the soil at each depth was determined. The results are given in Table I.

TABLE I.—*Effect of irrigation on arsenic in the soil, giving the percentage of arsenic at various depths*

Soil No. 1.			Soil No. 2.			Soil No. 3.		
Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.	Depth.	Not irrigated.	Irrigated.
Inches.	*Per cent.*	*Per cent.*	*Inches.*	*Per cent.*	*Per cent.*	*Inches.*	*Per cent.*	*Per cent.*
1 to 3	0.280	0.224	1 to 3	0.450	0.237	1 to 2	0.97	0.95
3 to 5	.198	.211	3 to 5	.170	.092	2 to 4	.50	.47
5 to 7	.171	.145	5 to 7	.118	.092	4 to 6	0	0
7 to 9	.184	.170	7 to 9	.013	.....	.....	.....	.....

The columns headed "Not irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were protected from rain and which received no irrigation. The columns headed "Irrigated" show the percentage of arsenic in the soil at the given depth in the lysimeters which were subjected to irrigation. A comparison of the two columns for each soil will show the strong fixing power of these soils for arsenic, the influence of different soil types upon the fixation, and the danger of its accumulation. Samples of soil No. 3 were taken at depths different from those of soils Nos. 1 and 2, as shown in Table I, because of the concentration of arsenic at the surface in the former.

In order to determine how nearly these results represent actual field conditions, samples of soil were obtained from a plantation at Nahiku, Maui, which was the first to adopt the use of sodium arsenite as a means of weed control. Weeds on this land have been sprayed for five years, at the rate of three applications per year, using 5 pounds of arsenic (As_2O_3) per acre for one application. During this time the soil has received no cultivation whatever and the rainfall averages about 200 inches per year. The soil is very porous and there is very little run-off water. Samples were taken at three depths: Every 4 inches of the first foot. The surface 4 inches contained 0.00924 per cent of arsenic (As_2O_3), and none was present below this depth. A determination made by boiling the soil with water showed an arsenic content of 0.00006 per cent, or 0.6 p. p. m., soluble in water. That the arsenic fixed by soils in the lysimeters was partly soluble in water indicates that the fixation is due in part to physical influences.

CHEMICAL REACTIONS INVOLVED IN THE FIXATION

The composition of the spray as prepared by recommended methods may be either a solution of the acid salt ($Na_2O.2As_2O_3.2H_2O$) or the neutral salt ($Na_2O.As_2O_3$), depending on the proportions of soda (either hydrate or bicarbonate) and arsenious acid used.

$$H_2O+2NaOH+2As_2O_3=Na_2O.2As_2O_3.2H_2O.$$
$$H_2O+2NaOH+As_2O_3=Na_2O.As_2O_3.2H_2O.$$

For the following experiments in studying the replacement phenomena, a solution of the neutral salt was used.

One liter of a 1 per cent solution of sodium arsenite was allowed to act upon 200 gm. of soil, with occasional shaking, for two weeks. Checks were also maintained with 200 gm. of soil and 1 liter of water. The arsenic extract was then separated from the soil and a partial analysis made to determine the elements with which the sodium arsenite is most active. The results are given in Table II, which shows the composition of a 1 per cent sodium-arsenite solution after contact with the soil, as compared with the solvent action of water. The percentage of humus

in the soil before and after treating with 1 per cent of sodium arsenite is also given.

TABLE II.—*Composition of the extracts (mgm. per liter)*

Constituent.	Soil No. 1.		Soil No. 2.		Soil No. 3.	
	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.	Water extract.	Arsenic extract.
Fe_2O_3	Trace.	716	Trace.	121	Trace.	90
CaO	11. 2	84	13. 6	124	74. 6	126
MgO	3. 6	20	10. 8	44	7. 4	26
As_2O_3	.....	3, 960	.....	6, 000	.....	4, 480
$Mg.As_2O_3$ fixed by 100 gm. soil	.....	2, 640	.....	600	.....	2, 120
Humus I[a], per cent	2. 77		1. 68		8. 75	
Humus II[a], per cent	1. 56		1. 80		8. 40	

[a] Humus I shows the percentage of the humus content of original soil; humus II, that of soil after treatment with the 1 per cent sodium-arsenite solution.

Table II shows a replacement of and a solvent action toward iron, calcium, magnesium, and humus, and suggests several theories as to the nature of the reaction. The soil absorbing the largest amount of arsenic lost through solution or replacement the most iron and humus. The soil absorbing the least arsenic lost the least iron and no humus. Apparently the absorption of arsenic by soil No. 3 is largely a mechanical fixation, as the data show a high absorption, but a low replacement.

In sodium arsenite we have the combination of a strong base with a weak acid. A well-known property of such salts is to react alkaline when dissolved in water. This is due to the faint dissociation of H_2O into H+ and OH− ions. Here the chemical and physical phenomena involved in the fixation of sodium arsenite are directly or indirectly a result of hydrolysis. The latter term as used herewith is intended to convey the increased dissociation in a solution of sodium arsenite, which itself is only faintly dissociated. This results in an increase in the concentration of the hydroxyl ion and the formation of the highly dissociated electrolyte sodium hydrate, which in the soil would probably be rapidly converted to bicarbonate. In this form it would have a solvent action toward the iron and humus and more or less toward the magnesium and calcium through the formation of slightly soluble bicarbonates. Magnesium bicarbonate is very unstable as compared to calcium bicarbonate and, hence, is precipitated following the solvent action of the sodium bicarbonate. The calcium is more soluble even in the soils containing much higher amounts of magnesium. These reactions leave the arsenic free as the negative ion to combine with the dibasic and tribasic metals to form slightly soluble arsenites or arsenates, thereby fixing the arsenic in the soil.

The rate and extent of fixation of arsenic vary in different soil types, owing to the concentration and solubility of the basic constituents—i. e., dissociation was found to be more rapid in some soils than others. To illustrate, the soil absorbing the greatest amount of arsenic exhibited the strongest alkalinity and showed the greatest chemical activity. Furthermore, this same soil contained the least amount of the soluble bases, calcium, magnesium, and potassium, indicating that the chemical fixation is influenced by the pressure of soluble bases.

SUMMARY

It has been shown herein that soils possess strong fixing power for arsenic and that when a sodium-arsenite spray is used for destroying weeds the arsenic will ultimately be deposited in the surface soil, there to remain in spite of the leaching effect of rains or irrigation.

The chemical reactions involved in the fixation are a replacement or solution of iron, calcium, magnesium, and humus, owing in part to a hydrolysis of the sodium arsenite in solution, also a combination with the dibasic and tribasic elements to form the difficultly soluble arsenites or arsenates.

ANGULAR LEAF-SPOT OF CUCUMBERS

By ERWIN F. SMITH, *Pathologist in Charge*, and MARY KATHERINE BRYAN, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

INTRODUCTION

The angular leaf-spot of cucumbers (*Cucumis sativus*) has been known in the field for many years, but up to the present time no organism has been named as its cause, though it has been generally conceded to be of bacterial origin. The disease is characterized by the formation of numerous, often confluent, angular, dry, brown spots which by dropping out or tearing give the leaves a ragged appearance.

The literature on the subject, aside from mere notes on the occurrence of the disease scattered through pathological literature, consists of four papers by O. F. Burger, of Florida,[1] and a more recent Italian paper by Traverso.[2] Burger mentions the leaf-spot as preliminary to a more destructive fruit-rot, said to be due to the same organism. His description of the diseased leaves agrees with the appearance of leaves sent to the writers from Wisconsin, as well as with those obtained by them from other States, and with the leaf-spots which they obtained in Washington by pure-culture inoculations. A brief description of the causal organism is given in each of his papers, in one case with the group number according to the chart of the Society of American Bacteriologists. Burger's descriptions agree in the main except as to flagella and the diameter of his organism. In his earlier descriptions it is said to have polar flagella, but in the later ones it is reported to be peritrichiate. No name is given to the bacillus.

Traverso's paper is only a preliminary one, but it leaves no doubt as to the identity of the Italian and American disease. A motile, fluorescent, nonliquefying organism was isolated by him and inoculations were made with it, but no positive results were obtained (p. 459).

Who first reported this cucumber disease in the United States is uncertain; the senior writer has known it for 20 years, and several years ago (1904) plated out two yellow bacteria with which unsuccessful inoculations were made. Again, in 1907, at his suggestion, Mr. John R. Johnston, then of the Laboratory of Plant Pathology, made platings

[1] Burger, O. F. A new cucumber disease. *In* Fla. Agr. Exp. Sta. Rpt. [1911]/12, p. c–ci. 1913.
——A bacterial rot of cucumbers. *In* Phytopathology, v. 3, no. 3, p. 169–170. 1913.
——Bacterial rot of cucumbers. *In* Fla. Agr. Exp. Sta. Rpt. [1912]/13, p. xc–xciv, fig. 11–13. 1914.
——Cucumber rot. Fla. Agr. Exp. Sta. Bul. 121, p. 97–109, fig. 37–42. 1914.

[2] Traverso, G. B. Sulla batteriosi del cetriolo in Italia. Nota preliminare. Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat., s. 5, v. 24, sem. 1, fasc. 5, p. 456–460. Apr. 5, 1915.

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and isolated a yellow schizomycete with which unsuccessful inoculations were made on cucumbers in the Department greenhouses.

ISOLATION AND IDENTIFICATION OF ORGANISM

Specimens were sent to the Laboratory of Plant Pathology in August and September, 1914, from New York and Wisconsin. No complaint was made by the sender of any association with fruit-rot, either on his own initiative or when questioned.

The interior of the spots was found to be swarming with bacteria which on floating out on the slide showed active motility. Plates were poured from such spots and a white, motile, rod-shaped organism was isolated. Spray inoculations with subcultures from three colonies on these plates gave typical infections on young cucumber leaves, from which the organism was reisolated. Colonies (subcultures) from this reisolation were then used for spray inoculations, and again the typical disease was produced with great virulence.

In August, 1915, specimens were received from several localities in Wisconsin, Indiana, and New York and from Ontario, Canada. In each case the same organism was isolated in pure cultures and used to produce typical infections on cucumber leaves in the hothouse.

The organism causing the angular leaf-spot of cucumbers appears to be an undescribed form for which the specific name *lachrymans* is suggested on account of the tearlike drops of exudate from the spots in early stages of the disease. Its brief Latin diagnosis is as follows:

Bacterium lachrymans, sp. nov.

Baculis cylindricis apicibus rotundatis, solitariis, saepe binis; baculis unis 0.8X1-2μ; 1-5 flagellis polaribus mobilibus; aerobiis, asporis.

Habitat in foliis vivis Cucumeris sativi in maculis angularibus. Liquefacit gelatinam lente. Colonae superficiales in agar-agar, rotundae, albae; colonae juvenes habientes centra non-translucida, et margines translucidas cum lineis multis radianti. bus. Lac sterile alkalinum et translucidum fit; casein non segregatur. Nitrum non redigitur; culturae in mediis cum saccharo sacchari et saccharo uvae acidae fiunt. Gas non facitur. Methodo Grami non coloratur.

The organism which the writers isolated from the Wisconsin cucumber leaves and have here designated "*Bacterium lachrymans*, n. sp." differed culturally is so many important respects from Burger's organism that all our cultural experiments were repeated. These repetitions, however, confirmed the differences, which are given in Table I.

While it is not doubted that Burger had this disease under observation, it is believed that the organism described by him is not its cause, but is rather the cause of a rapid soft-rot of the fruit. His organism, however, may be a wound parasite following injuries due to the organism here described.

TABLE I.—*Differences between Bacterium lachrymans and Burger's cucumber organism*

Bacterium lachrymans.	Burger's organism.
1. Polar flagellate	Peritrichiate flagellate.
2. Liquefies gelatin	Does not liquefy gelatin.
3. Clears milk without coagulation	Coagulates milk.
4. Strict aerobe (does not grow in closed end of fermentation tubes).	Facultative anaerobe (grows in closed end of fermentation tubes).
5. Forms acid from saccharose in fermentation tubes.	Does not form acid from saccharose in fermentation tubes.
6. Forms acid from dextrose in fermentation tubes.	Does not form acid from dextrose in fermentation tubes.
7. Not villous along line of stab in either agar or gelatin.	Villous along line of stab in both gelatin and agar.
8. Does not become yellow with age on sugar agars.	Becomes yellow with age on sugar agars.
9. Moderate indol formation	No indol formation.
10. Agar-plate surface colonies show many fine radiating lines.	Agar-plate colonies homogeneous in structure.
11. Does not cause soft-rot of cucumber fruits.	Causes a soft-rot of the fruit.
12. Surface colonies on agar plates are always round.	Agar colonies are round to ameboid.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Mr. Frederick V. Rand, of this laboratory, by whom these specimens were collected, reported the disease in 1915, from the following localities:

MICHIGAN: Big Rapids, Muskegon, Grand Haven, Holland, Grand Rapids, and Hudsonville.

INDIANA: Plymouth, Monterey, Tyner, and Donaldson.

WISCONSIN: Racine, Portage, Ripon, Princeton, and Milwaukee.

NEW YORK: Constable, Malone, North Lawrence, and Long Island.

CANADA: Provinces of Ontario and Quebec.

In regard to the amount of injury caused by this disease, Mr. Rand says:

In most cases I found the angular leaf-spot causing a rather minor injury, but in an occasional field I found all the leaves back of the tips of the vines very badly shot-holed and presenting an exceedingly ragged appearance, such that serious injury to the crop must inevitaby result. Last year this disease had done more damage than any other in the vicinity of Ripon, Wis.

This disease has also been reported recently from Maryland and several other Southern States.

Earlier the senior writer received specimens from Michigan, Wisconsin, Indiana, Connecticut, and the District of Columbia.

INOCULATION EXPERIMENTS

On October 26, 1914, young cucumber plants were sprayed in cages in the hothouse with water suspensions from young agar slants made from three colonies on the plates poured from diseased leaves. The plants were kept moist in the cages for 30 hours, then removed to the bench.

Five days later, water-soaked spots appeared on the leaves, and by November 3 there were typical browned spots on plants inoculated with each of the three colonies. These spots swarmed with bacteria. Poured plates on agar gave pure cultures of the same white organism. No further inoculations were made until April 30, 1915, when sprayings were again made in cages as before, using subcultures of colony No. 1, plated from a spot produced by the inoculations of October 26. The plants used in this case were of a common field variety and rather stunted but with sound leaves. Three days after the first spraying water-soaked spots appeared on the lower surface of the leaves, and by May 6 these had enlarged into the typical angular, dry, brown spots.

Another experiment on May 6, 1915, using perfectly healthy, free-growing Arlington white spine cucumber plants and subcultures from the same colony (No. 1) gave striking results. Several leaves showed tiny water-soaked areas on the second day, and all the leaves were typically and badly spotted by the sixth or seventh day. In this stage the spots were one-fourth to three-fourths of an inch in diameter, angular, following the larger veins, and water-soaked (translucent), not dry. In the early morning drops of moisture (exudate) swarming with bacteria were found hanging on the lower surface of such spots (Pl. XLV, fig. 1). Pure cultures of the causal organism were obtained by plating from one of these drops. On the following day, or even later on the same day, white films (bacterial crusts) replaced the drops (Pl. XLIII, fig. 1). The appearance of infected leaves at the end of 12 to 14 days, when the diseased areas have become dry and begin to drop out, is shown in Plate XLIII, figure 2.

As the young unsprayed leaves developed on these plants, they became naturally infected; and in three cases the stems and petioles of this young growth also became water-soaked, exuded drops of fluid (Pl. XLIV, X, X), and finally broke or bent over (Pl. XLV, fig. 2), ending the growth of the plant. The cracking open of stems in this stage of the disease is shown at X in Plate XLV, figure 2, and in detail in Plate XLV, figure 3.

On the green fruits up to the end of August, 1915, the writers were able, with one exception, to obtain within a week or 10 days (shipping time) only a local infection and a bacterial exudate such as that shown in Plate XLVI, figure 1—no general soft-rot. Even when the fruit (Pl. XLVI, fig. 1) was kept for another week at high temperatures (28° to 32° C.), it did not rot (Pl. XLVI, fig. 2). Altogether 15 such fruits were inoculated with virulent cultures, some on the vines and others in damp chambers.

Soft-rot occurred twice in young fruits (two-thirds grown) when placed in damp chambers after inoculation. In the first case (the exception referred to above), plates were poured from the soft interior of the one fruit thus affected. As only spreading fimbriate colonies were obtained, the soft-rot was attributed to an intruder, and no further studies were

made. Some months later (September, 1915) in a similar experiment two out of four inoculated fruits became soft-rotted. These fruits were from the market. All four showed the local gumming at the point of inoculation (needle pricks) after five days, while check pricks gave no gumming. Two days later two fruits began to soften, and the next day the whole interior was swarming with bacteria. Plates were poured from the interior of one of these fruits under sterile conditions, and again only spreading fimbriate colonies were obtained. Smears from these colonies stained by Van Ermengem's flagella stain gave rods with as many as 8 or 10 peritrichiate flagella. This organism grew well in the depths of agar stabs and curdled milk with reddening of litmus in milk. The other two inoculated fruits remained sound and after two weeks when cut open showed only a very local infection not extending much beyond the needle pricks in any direction.

Since the organism causing the leaf-spot is polar flagellate and aerobic, does not develop a fimbriate growth on agar, and does not curdle milk or redden litmus in milk, it is evident that this soft-rot was due to an intruder, which may have come from the surface of the fruits, since they were not sterilized, but only washed.

When these fruits became soft-rotted, the suspicion arose that possibly the softening and cracking of the stems and petioles (Pl. XLV, fig. 2) might also have been due to some unsuspected soft-rot organism. The inoculation experiments with *Bact. lachrymans* were therefore repeated on stems and petioles of free-growing cucumbers with the same result as before—i. e., softening and cracking of the younger stems and petioles. From one of these stems platings were made and *Bact. lachrymans* obtained in pure culture. At the same time several control inoculations were made on stems and petioles, using a subculture of the fimbriate, peritrichiate, soft-rot organism plated from one of the softened cucumbers above mentioned, but no rot occurred (four weeks). This organism, however, soft-rotted green cucumber fruits when inoculated by needle pricks.

Last of all, following the discovery of Traverso's paper, another set of inoculations was made on cucumber fruits. Six marketable green hothouse fruits were selected and inoculated with *Bact. lachrymans*. At the end of 10 days in culture dishes at temperatures varying from 24° to 30° C. all showed local gumming and infection about the needle wounds, but none of them developed any soft-rot (Pl. XLVI, fig. 3).

HISTOLOGY OF DISEASED LEAVES

Pieces of a leaf that showed spotting were fixed on the second day, embedded, sectioned, and stained. Stomatal infections were very numerous (Pl. XLVII, fig. 1). The bacteria gorged the opening of the stoma in some cases, as well as the cavity beneath it. Even at this early date the bacteria had spread in great numbers for some distance from the stoma, crowding apart or crushing the cells of the parenchyma and causing a slight swelling on the leaf (Pl. XLVII, fig. 2).

MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM LACHRYMANS

MORPHOLOGICAL CHARACTERS

As it occurs in the plant and also on media the organism causing the disease is a short rod with rounded ends, single or in pairs (Pl. XLVIII, fig. 2 and 3), 0.8μ wide by 1 to 2μ long. On culture media it occurs singly or in pairs with a very decided constriction, and occasionally (in salted bouillons) in chains of as many as 12 or more individuals (Pl. XLVIII, fig. 1). No spores have been seen. Capsules are formed on agar (Pl. XLVIII, fig. 2), and in milk (Ribbert's stain). It is motile by means of 1 to 5 polar flagella (Pl. XLVIII, fig. 3). It is Gram-negative and is not acid-fast.

EFFECT OF DESICCATION

When drops from 24-hour peptone bouillon were placed on sterile covers in sterile Petri dishes and kept in the dark at room temperature, the organism was not killed by 21 days' drying, but it gave no growth when covers were dropped into suitable bouillon after 6 weeks' drying.

TEMPERATURE RELATIONS

The best growth was obtained at 25° to 27° C. There was no growth at 36°, though bouillon was weakly clouded at 35° C. Slow growth occurred at 1° in bouillon cultures (two weeks' time).

SENSITIVENESS TO SUNLIGHT

Agar plates, thin-sown, from an 8-day bouillon culture were exposed, bottom up on ice, to sunlight in June for 5, 10, and 15 minutes, one-half of each plate being protected from the light by several thicknesses of black paper. After five days' incubation numerous colonies appeared, and no difference was observed between the insolated and covered side on any of the six plates (but the colonies were not counted). Another test was made in September, 1915, with the following results:

The fluid used for inoculation consisted of one 3-mm. loop from a 24-hour bouillon culture into 10 c. c. of bouillon. Five plates were inoculated, each with one 2-mm. loop from this suspension. Five other plates were inoculated, each with one needle from this suspension. One plate from each lot was then half covered and exposed bottom up on ice for 5, 15, 30, 45, and 60 minutes, respectively. Result: All were killed by 45 and 60 minutes' exposure; three-fourths were killed by 30 minutes' exposure; one-third were killed by 15 minutes' exposure; and one-fourth were killed by 5 minutes' exposure.

When these results were obtained with the 24-hour bouillon, the experiment with the 8-day bouillon was repeated. Four agar plates were poured, one-half of each being exposed bottom up on ice, two for 15 minutes and two for 30 minutes, the sky being clear and the sun bright (October 12).

There was a marked reduction of colonies on the plates exposed for 15 minutes (estimated, 70 per cent), and almost complete absence of colonies on those exposed for 30 minutes (estimated, 95 per cent destroyed). The contradictory earlier result must therefore be attributed to a feebly actinic condition of the sky not visible to the naked eye.

SENSITIVENESS TO FREEZING

The organism is quite sensitive to freezing. A transfer was made to beef bouillon from a 5-day-old bouillon culture, shaken well and allowed to stand for five minutes. Plates were then poured with measured loops from this culture. The tube was then buried in salt and pounded ice, frozen solid and kept frozen for 15 minutes, after which it was thawed in cool water (five minutes required), shaken thoroughly, and used for a second set of plates, the loops being measured exactly as before. Two days after pouring the colonies were counted. There were one-ninth as many colonies after freezing as before freezing (Pl. XLVII, fig. 3). A longer incubation (five days) did not increase the number of colonies on the plates.

Thinking that five minutes might not have been long enough to obtain a uniform diffusion of the bacteria in the fluid, the experiment was repeated, allowing the tube to stand an hour with shaking before the plates were poured. The result was practically the same, nine-tenths of the bacteria being destroyed by the short freezing, the count being made on the fifth day.

CULTURAL CHARACTERS

AGAR-POURED PLATES.—On +15 peptone-beef agar at 23° C. surface colonies 2 days old are 1.5 to 2 mm. in diameter, round, smooth, shining, slightly convex, finely granular (under the compound microscope), with an opaque white center and a thin, transparent, entire margin. When 3 to 4 days old at 23° C. the largest measure 4 to 7 mm. in diameter and the white opaque center spreads in radiating lines into the thin margin (Pl. XLIX, fig. 1). At higher temperatures (27° to 30° C.) they reach this size in two to three days. Buried colonies are lenticular. Later (when 4 to 5 days old) the surface colonies lose their dense white center and dry down very thin and transparent and then show little or no trace of the radiating lines.

AGAR STABS.—Stabs in +15 peptone-beef agar when 2 days old at 23° C. show a raised, smooth, shining, white, transparent, surface growth 8 mm. in diameter. Growth is visible only along the upper one-third of the stab. This is granular, not villous.

Old cultures have a thin white growth completely covering the surface, and the agar is then frequently pale green, fluorescent.

AGAR SLANTS.—On slant agar, stroke cultures make a moderate, thin, white, transparent, smooth, shining growth, denser in the center. There is considerable white sediment in the V.

GELATIN PLATES.—Surface colonies on gelatin plates show a peculiar margin, best seen under low magnifications, with oblique light (Pl. XLIX, fig. 2). Liquefaction is slow (18° to 20° C.), and when the layer of gelatin is thin (10 c. c. to a plate) does not take place, as the medium soon becomes too dry for growth. On plates containing 20 c. c. of gelatin liquefaction began on the twelfth day and on the sixteenth day was complete, the colonies floating intact in the liquid gelatin.

GELATIN STABS.—At 15° to 18° C. in +10 peptone gelatin the surface growth after seven days is about 6 mm. in diameter, with a pit of liquefaction 2 mm. wide and 2 mm. deep. Stab growth is granular, not villous, fading out downward. As liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-form (Pl. XLIX, fig. 3). Liquefaction progresses rather slowly but is complete within three to four weeks at the specified temperatures.

BEEF BOUILLON.—In +15 peptone-beef bouillon uniform clouding occurs within 24 hours. This clouding is weak to moderate, never strong. On the second day a membranous pellicle is formed, which fragments and falls readily on shaking. It is made up of a homogeneous mass of bacteria—i. e., free from pseudozoogloeæ but containing a few short chains (10 or 12 individuals). Old cultures (4 to 6 weeks old) are often decidedly green fluorescent. The white precipitate breaks up readily on shaking and contains many small crystals.

POTATO CYLINDERS.—When inoculated from agar cultures growth on steamed potato cylinders in two days is moderate, spreading, creamy white, shining, and slimy. The part of the potato out of the water becomes slightly browned. Growth on potato soon ceases. After 10 days the color of the potato is completely changed, becoming a pale brownish hue, and the growth takes on a similar color (very pale brownish). Tested with alcohol iodin for starch, such cultures give a heavy dark-purple reaction, showing that there has been only a partial digestion of the starch (formation of amylodextrin). The cylinders are not softened.

MILK.—Inoculated milk clears slowly and without coagulation. Clearing begins within a week, and after two weeks tubes of it are translucent so that the outlines of a pencil back of the milk may be seen through it clearly. Cultures 1 month old are still clear but are then tawny olive,[1] with a darker rim where the milk has dried down.

LITMUS MILK.—Lavender-colored litmus milk begins to blue from the top downward on the second day and is completely blued by the third day, without a sign of coagulation or clearing. A decided creamy-white pellicle is formed.

After 10 days clearing begins and is complete in 20 days. Later the blue color bleaches out (reduction phenomena), beginning at the bottom, leaving the whole fluid a clear (translucent) brown. At no time is there any reddening of the litmus or any coagulation of the milk; nor are any crystals formed in it.

FERMENTATION TUBES.—The tests in fermentation tubes were made in water containing 2 per cent of Witte's peptone, to which was added 2 per cent of the carbon compound to be tested—namely, saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open end of each on the second day, heaviest in the tubes containing saccharose and dextrose, but the closed end in every case remained clear, with a distinct line across the inner part of the U. When 5 days old they were tested with neutral litmus paper. Saccharose and dextrose gave a decidedly acid reaction, while all the others were neutral. When 20 days old the saccharose and dextrose were still acid and the others weakly alkaline. No gas was formed and no growth occurred in the closed end of any.

No gas was formed in fermentation tubes containing sterile milk; nor was there any separation of the curd. The milk in the open end cleared gradually, while that in the closed end remained unchanged. The litmus reaction was alkaline in the open end.

Nitrate bouillon in fermentation tubes gave a good clouding in the open end, none in the closed end, no gas, and no nitrate reduction. A decided alkaline reaction was obtained with neutral litmus paper.

TOLERATION OF SODIUM CHLORID.—Neutral peptone-beef bouillons containing 2, 5, 6, and 7 per cent of chemically pure sodium chlorid, respectively, were inoculated from young bouillon cultures. Growth was retarded by 2 per cent of sodium chlorid

[1] Ridgway, Robert. A nomenclature of colors . . . 129 p., 17 pl. (partly col.). Boston, 1886.

and inhibited by all the other strengths. The experiment was repeated using 2, 3, and 4 per cent of sodium chlorid. Again, the 2 per cent retarded growth (clouding on the fourth day). Checks clouded after 24 hours. Growth appeared in the 3 per cent after 12 days, but there was no growth in the 4 per cent even at the end of four weeks. In both 2 per cent and 3 per cent the growth was scanty and flocculent, composed largely of chains (Pl. XLVIII, fig. 1), especially in the 3 per cent solution.

TOLERATION OF ACIDS.—Neutral bouillon containing 0.1, 0.2, and 0.3 per cent, respectively, of malic acid, tartaric acid, and citric acid was used. After three days the 0.1 per cent cultures of all three acids were well clouded; the 0.2 per cent malic and tartaric acids were all moderately clouded, while the 0.2 per cent citric acid showed no growth. None of the 0.3 per cent cultures were clouded. After three weeks the 0.2 per cent citric acid was well clouded, but in no case did the 0.3 per cent cultures show any growth. The cultures were watched for five weeks.

TOLERATION OF ALKALI.—The organism is quite sensitive to alkali. Peptonized beef bouillons titrating, according to Fuller's scale, +25, +20, +10, +5, 0, −5, −20, and −30, were inoculated from a 4-day bouillon culture, using a carefully measured 3-mm. loop for each tube. After 24 hours all showed growth except the −20 and −30. Heaviest growth occurred in the +25, weakest growth in the −5, which was flocculent instead of clouded. Five days later the same relative growth was evident throughout the series, but the −5 had become clouded and the −20 weakly flocculent. The −30 remained clear. After two weeks there was moderate growth in the −20, but none in the −30. The alkali used was sodium hydrate.

USCHINSKY'S SOLUTION.—In Uschinsky's solution growth is heavy, with a heavy membranous pellicle which falls readily as a whole. Greening of the media begins at the top on the second or third day and proceeds rapidly downward until the whole is a decided pale apple green. The medium does not become viscid.

FERMI'S SOLUTION.—At the end of 10 days a fine green fluorescence like that in Uschinsky's solution is visible. No fluorescence appeared in tubes of Cohn's solution inoculated on the same date for comparison.

COHN'S SOLUTION.—There is good clouding, heaviest near the top, but without a pellicle. Numerous floating crystals occur and the white precipitate is dotted with crystals. No greening occurs.

SUGAR AGARS.—No yellowing occurred on any of the sugar agars used. Cultures were made on beef-peptone agars containing, respectively, 2 per cent of saccharose, maltose, and dextrose, and in sugar agar without beef—i. e., containing only peptone and saccharose. The cultures were watched for eight weeks, during which time they remained white.

DOLT'S SYNTHETIC AGAR.[1]—Growth is abundant, covering the surface on the third day with a thin pink layer. Reddening of the dark agar begins on the second or third day; and after 10 days the color is changed throughout, although the lower half has not lost completely its purplish hue.

BOUILLON OVER CHLOROFORM.—Growth is not retarded in unshaken tubes of peptone-beef bouillon to which 5 c. c. of chloroform have been added.

REDUCTION OF NITRATES.—Nitrates are not reduced. Five-day-old cultures in nitrate bouillon were tested by the addition to each of 1 c. c. of boiled starch water, 1 c. c. of potassium-iodid water, and 10 drops of sulphuric acid. There was no color reaction.

INDOL.—There is a weak indol production in 2 per cent peptone water and in peptonized Uschinsky's solution. Tests were made at the end of the fifth and tenth days by the addition of 1 c. c. of the standard sodium-nitrite solution and 10 drops of the sulphuric-acid water to each tube. No reaction appeared until the cultures were heated to 70° C., when a feeble but decided pink color appeared. The checks gave no pink reaction. A better reaction was obtained in peptone water containing 0.5 per cent of sodium chlorid (Dunham's solution)—about one-third that of *Bacillus coli*.

[1] Contains litmus, glycerin, milk sugar, and dibasic ammonium phosphate.

HYDROGEN SULPHID.—Strips of filter paper soaked in strong lead-acetate solution and dried were suspended over cultures in peptone-beef bouillon, milk, steamed potato, carrot, and turnip. No browning of the paper occurred within six weeks.

METHYLENE BLUE IN MILK.—Methylene blue is rapidly reduced. Cultures were made in milk containing 4 per cent of a 1 per cent solution of methylene blue. Bleaching begins on the second day and is complete or nearly so in six days, except for a pale-blue surface layer 2 to 4 mm. deep and a deep-blue rim and pellicle. This pellicle, when examined under the microscope, is seen to be composed of masses of bacteria that have taken up the stain. When shaken repeatedly, these bleached cultures regain their blue color.[1]

BLOOD SERUM.—Stroke cultures on Loeffler's blood serum give a moderate, white, shining filiform growth 3 mm. wide. There is no liquefaction even after eight weeks and no color change in the substratum.

AEROBISM.—The organism appears to be strictly aerobic. It does not grow in the closed end of fermentation tubes with any carbon food tested. In agar stab cultures no growth occurs in the lower end of the stab. Cultures were also made by shaking an inoculated tube of melted agar, but no growth occurred more than 3 mm. below the surface. Stabs were made in agar, then 10 c. c. of melted agar poured on top. No growth occurred in the stab or at the junction point, but there was good growth on the exposed surface of the added agar.

LITMUS AGAR WITH SUGARS.—On litmus-lactose-agar stroke cultures there is moderate growth and no color change.

Stroke cultures on litmus-maltose agar give heavy growth, but do not alter the color.

On litmus-saccharose agar growth is heavy and the medium reddens, beginning at the thin upper end. The reddening begins on the second or third day and is complete on the fifteenth day.

Following the chart of the Society of American Bacteriologists, the group number is 211.23221*23.

EFFECT OF COPPER SULPHATE ON THE ORGANISM

Bouillon cultures 24 hours old were exposed to the action of chemically pure copper sulphate in the following manner. A dilution of copper sulphate (1 to 1,000) was made in a large Jena flask and allowed to stand overnight. After shaking thoroughly, further dilution was made again (in liter quantities) to 1 to 100,000 and 1 to 500,000. After these had been well shaken and had stood for an hour 10 c. c. of each were put into sterile test tubes and a loop of a well-clouded suspension from a 24-hour-old agar culture was added. Plates were poured after 5, 10, 20, and 30 minutes from each tube, using carefully measured loops. Checks were made by pouring plates with the same measured loops from a similar dilution in sterile water.

The plates were incubated at room temperature (27° to 30° C.). A colony count was made on the second day. Exposure to the 1 to 500,000 dilution gave no observed reduction of colonies, but the 1 to 100,000 destroyed nine-tenths of the organisms. The experiment was repeated with a strength of 1 to 50,000 of copper sulphate. All were killed at this exposure, while the check gave numerous colonies.

[1] The blue pigment is also absorbed by the bacteria from peptone water containing methylene blue.

*Nonchromogenic on most media, but green fluorescent in Uschinsky's solution, Fermi's solution, and old peptone-beef bouillon.

Some weeks later the experiment with copper sulphate was repeated. To liter quantities of distilled water in Jena flasks, chemically pure copper sulphate was added so as to obtain the following dilutions: 1 to 50,000; 1 to 100,000; and 1 to 500,000. Some hours after full solution, 10 c. c. of each dilution were pipetted into sterile test tubes and to each was added a 3-mm. loop from a heavily clouded water suspension made from a 24-hour agar slant culture. From each of these tubes three plates were then poured at the end of 5 minutes, and again three more at the end of 10 minutes. As a check, a 3-mm. loop of the cloudy bacterial suspension was added to 10 c. c. of distilled water and from this tube three plates were also poured. The agar for the first set of poured plates was seeded with a 3-mm. loop from the dilution tube, that for the second set with a 2-mm. loop, and that for the third set with a needle dipped one-half inch into the fluid. The results in colonies are given in Table II, the counts being made on the sixth day.

TABLE II.—*Effect of copper sulphate on Bacterium lachrymans*

Dilution used.	Number of colonies of *Bacterium lachrymans* developing in—						
	Checks.	1 to 50,000 copper sulphate.		1 to 100,000 copper sulphate.		1 to 500,000 copper sulphate.	
		5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.
Plate 1 (3-mm. loop)..	3,844	78	45	118	55	3,412	1,756
Plate 2 (2-mm. loop)..	2,296	27	16	29	44	2,400	916
Plate 3 (needle).....	22	0	0	0	0	12	5

SUMMARY

(1) The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States.

(2) It is characterized by angular brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish.

(3) Young stems and petioles may become soft-rotted or cracked open.

(4) A virulent outbreak often materially reduces the crop by destroying the needed active leaf surface.

(5) The spot is caused by *Bacterium lachrymans*, n. sp., which enters through stomata, no wounds being necessary. This organism is quite different from the one described by Burger[1] in his papers on cucumber rot. No direct connection has been found between the leaf-spot and the soft-rots of the fruit.

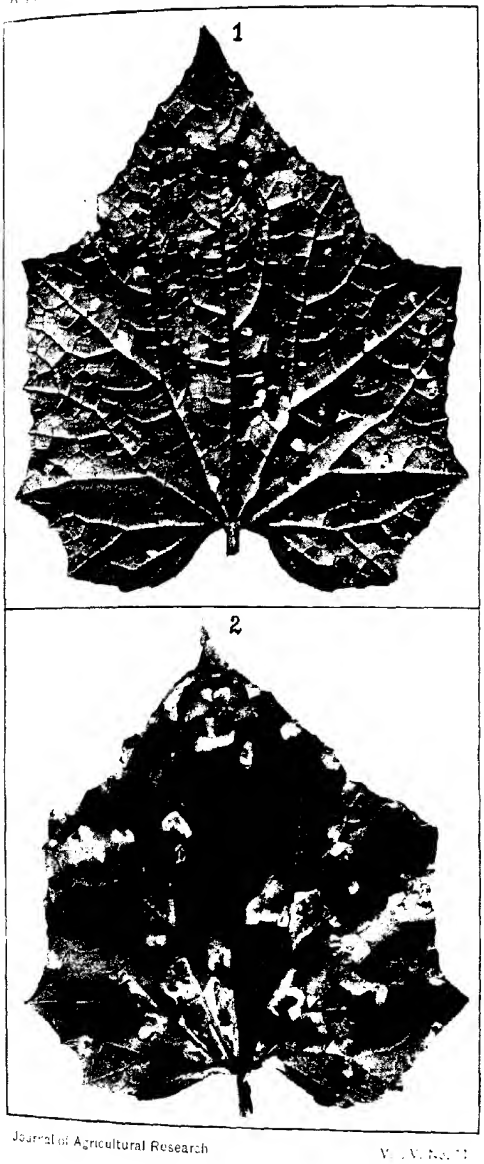
(6) Considering the results obtained in the laboratory with copper sulphate, it would seem that Bordeaux mixture properly applied is the remedy for this disease. Thorough field tests with it should at least be undertaken where the disease is troublesome.

[1] Burger, O. F. Op. cit.

PLATE XLIII

Fig. 1.—Cucumber leaf eight days after inoculation with *Bacterium lachrymans*. The bacterial exudate has now dried down into white crusts.

Fig. 2.—Cucumber leaf 12 days after spraying with *Bact. lachrymans*. Diseased tissue shriveled and spots falling out.


1
2


X
X

PLATE XLIV

Cucumber stem diseased by *Bacterium lachrymans*. The white bacterial exudate may be seen at X, X. Photographed 14 days after spraying.

PLATE XLV

Fig. 1.—Fragment of a cucumber leaf showing angular leaf-spots due to pure-culture inoculation with *Bacterium lachrymans*. Time, six days. The glistening tearlike exudate can be seen in a number of places. × 2.

Fig. 2.—Cucumber plant 18 days after spraying with *Bact. lachrymans*. Upper part of stem softened and shriveled. Lower part as at X with canker-like cracks which show bacterial exudate.

Fig. 3.—Stem at X in figure 2 enlarged to show bacterial lesions.


1
2
X
3

Angular Leaf-Spot of Cucumbers

PLATE XLV

PLATE XLVI

Fig. 1.—Green cucumber fruit photographed six days after inoculation with *Bacterium lachrymans*. There is an exudate at the point inoculated (upper part of fruit), while the remainder of the fruit is sound.

Fig. 2.—Same fruit as shown in figure 1, but at the end of 12 days. The fruit, which was slowly ripening, was still sound both externally and within, except at the point inoculated.

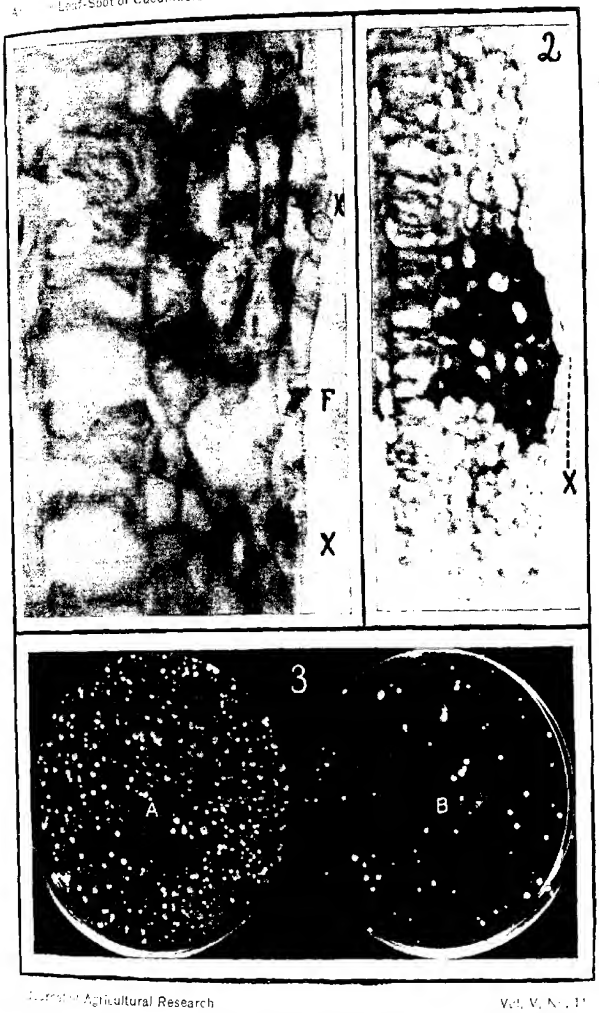
Fig. 3.—Section of green cucumber fruit 10 days after inoculation with *Bact. lachrymans* (6 days at 24° and 4 days at 30° C.). Not from the same series as figures 1 and 2. Tissue decayed only in the vicinity of the needle wounds.

PLATE XLVII

Fig. 1.—Cross section of a cucumber leaf, showing two stomatal infections (X, X. At *F* there is a third stoma whose chamber is free from bacteria. Stained with carbol fuchsin. × 1,000, nearly.

Fig. 2.—Cross section of cucumber leaf showing a dense bacterial infection due to *Bacterium lachrymans*. Stoma at X. Moderate magnification. Carbol-fuchsin stain. Tissues pushed out.

Fig. 3.—*A*, Agar-poured plate from bouillon dilution of *Bact. lachrymans*; *B*, agar-poured plate made from same quantity of same bouillon as *A*, but after freezing 15 minutes.


X
F
X
2
X
3
A
B

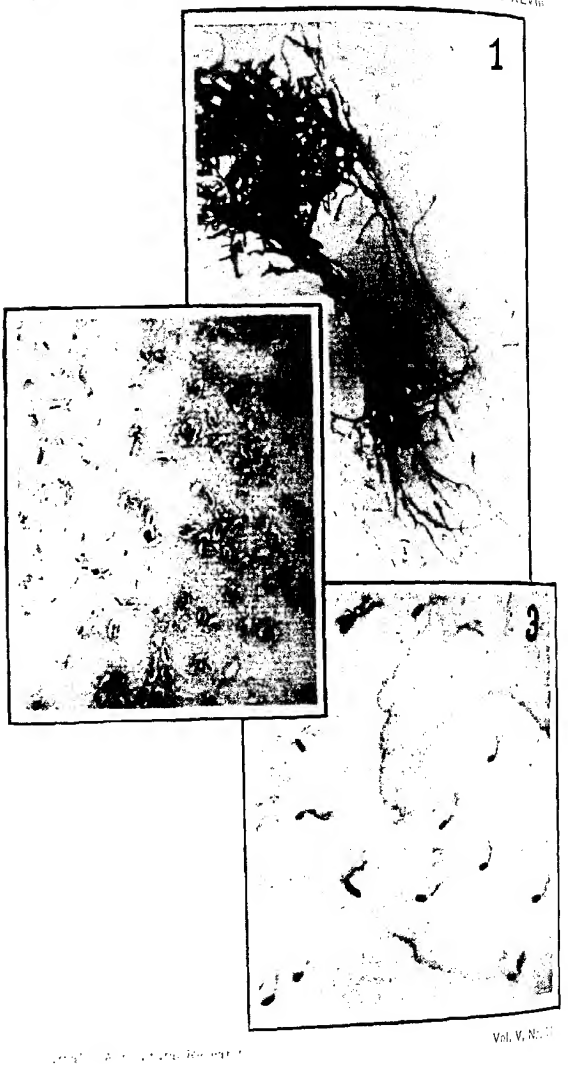

1
3

PLATE XLVIII

Fig. 1.—Chains of *Bacterium lachrymans* from 14-day-old culture in salted bouillon. Stained with carbol fuchsin. × 1,000.

Fig. 2.—Capsules of *Bact. lachrymans* from young agar culture. Ribbert's capsule stain. × 1,000.

Fig. 3.—Flagella of *Bact. lachrymans* from 24-hour-old agar slant. Stained by Van Ermengem's silver-nitrate method. × 1,000.

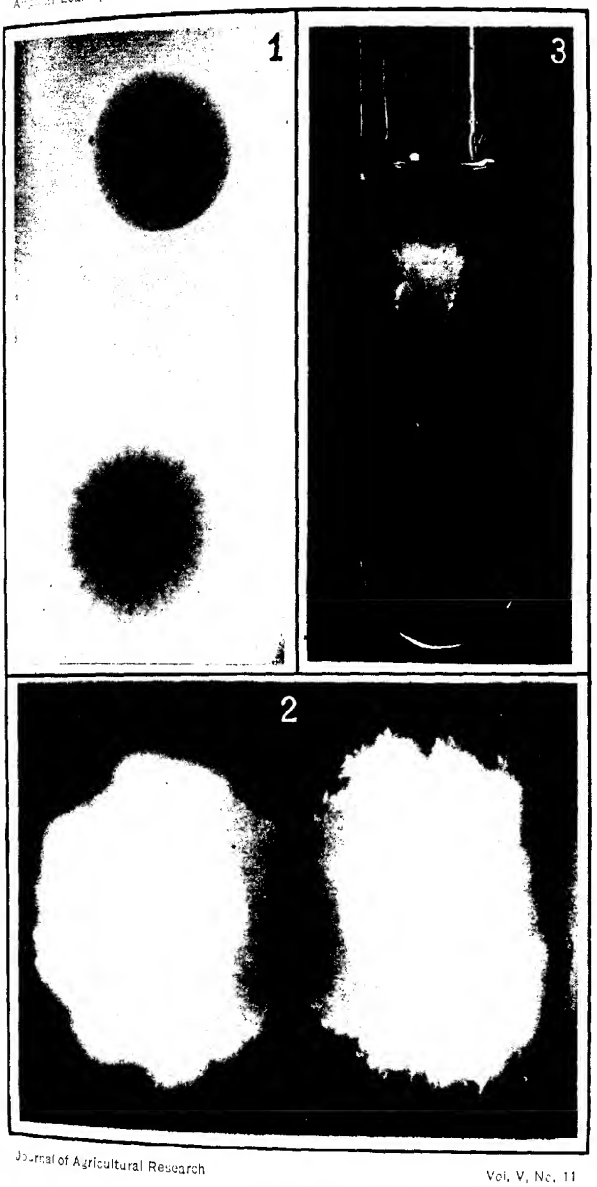
12571°—15——3

PLATE XLIX

Fig. 1.—Young surface colonies of *Bacterium lachrymans* on agar poured plate, showing opaque center and lines radiating into the thinner margin. × 14.

Fig. 2.—Surface colonies of *Bact. lachrymans* on gelatin poured plate. Photographed to show characteristic margin. × 14.

Fig. 3.—Gelatin stab culture of *Bact. lachrymans*, kept at 20° C. and photographed at the end of 12 days. Liquefaction confined to the top, but a discrete growth along the line of the stab nearly to the bottom of the tube.


1
3
2

ACTIVITY OF SOIL PROTOZOA[1]

By GEORGE P. KOCH,

Research Fellow, the New Jersey State College for the Benefit of Agriculture and Mechanic Arts

INTRODUCTION

The belief that soil protozoa are destructive to bacteria and, hence, are influencing factors in soil fertility is encouraging the more extended study of these organisms. It was shown elsewhere (5)[2] that the soil contains many cysts of protozoa which become active under favorable conditions. To serve as limiting factors in the soil, protozoa must be present in the active condition, for it is only as such that they can destroy bacteria and other micro-organisms; thus, the question at once presents itself, Are the protozoa active in the soil?

In 1909 Wolff (13) recorded investigations with soil protozoa undertaken for the purpose of ascertaining whether these organisms lead an active life in the soil and of discovering the factors which influence their activity. As to the presence of protozoa in the soil, Goodey (2), in 1911, concluded that they were not active in normal soils. A few years later, however, he (4) found that ciliated protozoa are in the encysted condition and concluded that the amebæ and flagellates were the limiting factors in the soil. Martin and Lewin (7) upon examining cucumber-sick soils found several different kinds of protozoa. The amebæ were probably the dominant type, and the flagellates were comparatively few. In 1911 Russell and Golding (9) noted that species of Vorticella, Putrina, Euglena, and other types present in ordinary soils were also found in sewage-sick soils. These organisms were more active in the sewage-sick soil than in ordinary field soil. In 1913 Russell and Petherbridge (11), in studying "sickness" in cucumber soil, found it to be full of organisms like myxomycetes, active amebæ, eelworms, and other lower animal forms.

Sherman (12, p. 630), who studied the presence of protozoa in several types of soil, summarizes his observations as follows:

> Certain forms of the soil protozoa are active under normal, and even sub-normal, conditions of moisture. The active protozoan inhabitants of most soils are probably restricted to flagellates. *Colpoda cucullus* is probably active whenever the moisture content is much above normal but does not appear to be so ordinarily.

[1] Contribution from the Laboratories of Protozoology, Soil Bacteriology, and Soil Chemistry of the New Jersey Agricultural College and Experiment Station.

[2] Reference is made by number to "Literature cited," p. 488.

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N. J.—3

As to the activity of soil protozoa, Cunningham (1, p. 56) states:

> To the question as to whether the protozoa lead an active life in the soil, it has been shown that the action of heat combined with the dilution method does not give a definite answer. That question, however, is answered in the affirmative by the results of experiments which will now be discussed.

Martin and Lewin (8, p. 117) likewise in a recent article concluded that "it seems probable from the work that we have done up to the present that there are always some free living protozoa present in a trophic state in even relatively dry, poor soils."

In this study it is the purpose of the writer—

(1) To develop a method for studying protozoan activity in the soil.

(2) To ascertain whether the protozoa lead an active life in soils of different moisture content when the temperature is constant and when it is variable.

(3) To study the effect of moisture on the activity of the protozoa in the soil under constant and variable temperatures.

(4) To study the length of the period of excystment of soil protozoa.

METHOD FOR STUDYING PROTOZOAN ACTIVITY IN THE SOIL

In studying the activity of protozoa in the soil the first difficulty which is encountered is the lack of a suitable method by which the investigator can determine with certainty the extent to which these organisms are active in the soil. Several methods are recorded that have been used with more or less success. In 1911 Goodey (2) passed an electric current through the medium and found that the living protozoa traveled with the current to the cathode. The separation of active forms by centrifugation was attempted by Russell and Golding (10) in 1912. In 1913 Martin (6) discussed a simple method based on the mixing of a small quantity of soil with picric acid and then noting the organisms (bacteria, protozoa, and diatoms) which rose to the surface, when this mixture was placed in a wide dish and the soil stirred. Cunningham (1) employed the dilution method for examining and counting the protozoa in the soil. Martin and Lewin (8) discuss several methods which they have employed with more or less success. For the detection of living amebæ, an air-blast method which they have devised has proved to be the most successful.

It was suggested by Martin and Lewin (8, p. 110) that—

> Any method which depends upon the addition of water to the soil must admit of very rapid execution, otherwise there is danger of protective cysts present in the soil opening, and thus giving a false impression as to the constitution of the active fauna. This danger is probably a very real one in the case of small flagellates, and especially the resting forms of some green algæ, in the case of which a few minutes' immersion in water may make the difference between a resting and an active form.

In order to determine the presence of motile protozoa in the soil, the writer has found the direct method of examining the soil to which a little water has been added the most satisfactory.

Several drops of sterile tap water (15 pounds' pressure for 15 minutes) are placed on a clean slide; then by means of a stirring rod a small portion of soil is stirred in this water and spread out in a thin film, so that the observer can readily see between the soil particles. Examinations are then quickly made under the low power (16 mm. lens) of the microscope.[1] As soon as the soil touches the water, the time is recorded and the examination is continued for a period of not more than two minutes, in this way reducing the possibility of error which the observer might make on account of the rapid excystment of the protozoa, as was suggested above.

PROTOZOAN ACTIVITY IN SOILS OF DIFFERENT MOISTURE CONTENT AND UNDER CONSTANT AND VARIABLE TEMPERATURES

GREENHOUSE SOILS

The conclusions of other investigators as to the presence of protozoa in the active state in normal soils led the writer to examine greenhouse and field soils for the purpose of finding out, if possible, to what extent the protozoa were present in the active state in the different soils.

Twenty greenhouse soils of different composition and texture were examined, each for half an hour, a new sample being placed on the slide every two minutes. These samples were all taken at a depth of 1 inch from the surface. The examinations were all made in the greenhouse. The results are given in Table I.

From Table I it is seen that protozoa can and do exist in the active state in greenhouse soils. Their presence, however, is very limited, as they were found in but 6 out of the 20 soils examined. All the soils in which the protozoa were found were of open structure and their moisture content was much above their optimum. A compact shale soil with added manure and high moisture content did not show any living protozoa. Soils with a large proportion of organic matter and with a relatively low percentage of moisture did not seem to encourage the presence of active protozoa. From the data presented it would seem that the moisture content is the primary limiting factor, while the texture and content of organic matter are secondary.

[1] In studies previously recorded (1), all the examinations were made under the low power of the microscope, as it was not possible to distinguish between motile bacteria and what might be called "protozoa." In the studies referred to, no difficulty was encountered in seeing protozoa which were as small as species of Bodos or Monos; hence, the data collected in this study are based on the examinations made under the low power of the microscope.

TABLE I.—*Extent of protozoan activity in greenhouse soils*

Laboratory No.	Kind of soil.	Fertilizer treatment.	Temperature.	Moisture content.	Presence of protozoa.[a]
			°C.	*Per cent.*	
1	Clay loam.....	20 per cent of compost + minerals.	20. 8	26. 65	
2	Shale.....	20 per cent of compost.....	20. 9	34. 30	
3	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 0	26. 66	S.C.† A.†
4	Sandy.....	20 per cent of compost.....	21. 0	26. 84	
5	Clay loam.....	40 per cent of compost.....	24. 0	36. 27	
6	Shale.....	20 per cent of compost; 30 per cent of sand.	22. 7	25. 17	
7	Sandy loam....	No mixture.....	21. 6	22. 59	S.C.†
8	Clay loam.....	20 per cent of compost; 20 per cent of sand.	21. 1	27. 57	
9	.....do.....	40 per cent of compost.....	21. 1	35. 75	
10	Sandy.....	20 per cent of compost.....	20. 8	26. 59	S.C.†
11	.....do.....	40 per cent of compost.....	23. 0	35. 35	S.C.† F.†
12	Sandy loam.....	.....do.....	22. 7	31. 28	
13	.....do.....	20 per cent of compost.....	22. 5	29. 10	S.C.†
14	Clay loam.....	20 per cent of compost + minerals.	24. 6	27. 90	S.C.†
15	Shale.....	20 per cent of compost + 10 per cent of sand.	21. 0	31. 75	
16	Clay loam.....	No mixture.....	19. 0	26. 21	
17	.....do.....	20 per cent of compost + minerals.	20. 3	31. 07	
18	Sandy loam....	20 per cent of compost.....	24. 0	25. 81	
19	Clay loam.....	20 per cent of compost; 20 per cent of sand.	24. 6	25. 09	
20	.....do.....	No mixture.....	18. 0	26. 60	

[a] S.C.=small ciliates; L.C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

FIELD SOILS

The extent of protozoan activity in field soils was studied in the same manner as the greenhouse soils. Samples of 14 field soils of different texture and tillage treatment were collected at a depth of 3 inches from the surface and brought to the laboratory in flasks. The temperature was in all cases noted. These were examined at once, each for half an hour, a new sample being placed on the slide every two minutes, as in the case of greenhouse soils. The moisture content was likewise determined. The soils were sampled and examined under normal conditions, again two days after a fall of 1.69 inches of rain, and a third time five days after 1.69 inches of rainfall. The second sampling was made at that period, since it allowed the organisms sufficient time to excyst, if possible, when the moisture content of the soil was increased. Likewise, the third examination was made five days after the heavy rainfall, for if the protozoa excysted and were washed to a lower level in the soil, this lapse of time allowed them to return to their normal level in the soil. Each soil was subjected to a half-hour's examination at every

sampling. In order to ascertain whether the soils contained cysts of protozoa which would become active when conditions became favorable after they had been examined, the soils collected at the third sampling were water-logged with sterile tap water and allowed to stand in the laboratory for 40 hours, when they were examined for motile protozoa. (See Table II.)

TABLE II.—*Extent of protozoan activity in field soils under different conditions of moisture*[a]

Laboratory No.	Kind of soil.	Soil treatment.	Normal moisture content.	Moisture content two days after heavy rain.	Moisture content five days after heavy rain.	Presence of active protozoa when soil samples were water-logged.[b]
			Per cent.	*Per cent.*	*Per cent.*	
1	Shale.....	Bare.....	25. 07	22. 01	21. 73	S.C.††† F.†
2	Sandy loam.....	Orchard...	13. 73	18. 66	14. 18	S.C.†† F.††
3	Gravelly sandy loam.	Garden....	9. 62	12. 40	8. 60	S.C.† F.†
4	Clay loam.....	Orchard...	15. 14	19. 18	12. 72	S.C.† F.†
5	Gravelly clay...	Meadow...	15. 67	20. 10	15. 21	S.C.††† L.C.† F.†
6	Clay loam.....	...do.....	19. 65	17. 30	14. 88	S.C.† L.C.† F.†
7	Silt loam.....	Wheat.....	11. 22	15. 24	9. 38	S.C.†† F.†
8	.....do.....	Weeds.....	13. 42	16. 08	14. 62	S.C.†† L.C.† F.†
9	Sandy.....	Corn.....	11. 34	14. 88	11. 25	S.C.†† F.††
10	Gravelly silt loam.	Fallow.....	10. 93	14. 28	10. 58	S.C.†† F.†
11	Shale.....	Bare.....	19. 88	23. 36	20. 27	S.C.††† L.C.† F.†
12	Gravelly silt loam.	Wheat.....	9. 60	15. 51	8. 97	S.C.† F.†
13	Silt loam.....	Corn.....	10. 90	15. 66	10. 95	S.C.†† F.††
14	Sandy loam.....	Vetch and tomatoes.	6. 74	12. 18	8. 52	Do.

[a] Under normal conditions and two and five days after a heavy rain no active protozoa were observed.
[b] S. C.=small ciliates; L. C.=large ciliates; F.=flagellates; A.=amebæ; †=few; ††=several; †††=many.

The careful examination of the 14 soils in no case revealed any motile protozoa, indicating that under the normal and even somewhat abnormal conditions of moisture active protozoa did not seem to be present in the soils examined. Several samples of standing rain water were collected when the second and third samplings were made. Upon examination all of the samples of water showed the presence of many small ciliates and flagellates, which indicates that the protozoa are active in accumulated water. In all cases where the 14 soils were water-logged small ciliates and flagellates, and in some cases even large ciliates, were present in the active state. The data presented in Table II point to the fact that all ordinary soils contain cysts of protozoa, and in the 14 soils examined the active organisms were not observed until sufficient moisture was present. It would seem that if the protozoa did become active when the moisture content was higher than it was at the time of the first sampling after the heavy rain, they remained active but a very short period of time, as in no case were they

found in the living condition, while in soils of very open structure where little or no surplus water is available they would seldom, if ever, become active. This point requires further investigation.

The question at once arises, How are protozoan cysts transported to the different soils? This process is likely to be brought about by wind action, by flowing water, and by mechanical means in the case of cultivated soils. Likewise, if the protozoa do not exist in the active state in the soil, can they and do they multiply? Under certain abnormal conditions of moisture they will become active and remain active as long as there are sufficient moisture and food and the absence of toxic or decomposition products. During this period multiplication takes place. When the conditions become unfavorable, no doubt some die, while the greater number encyst until conditions again become favorable for them to become active.

EFFECT OF MOISTURE ON THE ACTIVITY OF PROTOZOA IN THE SOIL UNDER CONSTANT AND VARIABLE TEMPERATURES

Large samples of three soils which had previously been used by the writer (1) in his study of protozoa were collected. The first was a 20 per cent manure shale, greenhouse soil, the second, a clay loam orchard soil which had received no applications of manure for the last 20 years; and the third, a sandy loam field-plot soil that for a period of 20 years had been receiving annual applications of manure at the rate of 20 tons per acre. (Hereafter throughout this study the first soil will be designated as the "greenhouse soil," the second as the "orchard soil," and the third as the "field soil.") The soils were air-dried at laboratory temperature and then sieved through a 20-mesh sieve. The optimum moisture content of these soils was determined. Twenty 50-gm. portions of each soil were weighed into 4-ounce bottles. With each soil one series of five samples was left air-dried. To one series sufficient sterile tap water was added to make the moisture content half of the optimum. To another series enough water was added to increase the water content to the optimum. To a fourth series sterile tap water was added so that the resulting mixture would be equivalent to one and a half of the optimum. At one and one-half of the optimum the soils could take up all the moisture without any free water being present. The soils were well mixed with a stirring rod, so that the moisture content was homogeneous throughout. In order to prevent condensation on the sides of the bottles, they were left unplugged. The flasks containing four samples of each soil, representing four moisture contents, were incubated at 5° to 7° C., one series at 15° to 17°, one at 22° to 24°, one at 32° to 33°, and one at the outdoor temperature. The samples were weighed daily, and the slight amount of moisture lost by evaporation was replaced. Each sample of soil was then examined for active protozoa not fewer

than three times, a new sample being taken every two minutes; during the examinations the respective samples were kept at the different temperatures. Sterile tap water of the same temperature as that at which the respective soils were incubated was used in making the examinations. Each series of samples were kept screened from the light during the period of incubation. After examination the samples were again weighed to determine the quantity of soil used in examination. Daily examinations of each sample of each soil were made for a period of eight days. (See Table III.)

TABLE III.—*Presence of active protozoa in different soils, with varying amounts of moisture at different temperatures (constant and variable) for a period of eight days*

Laboratory No.	Kind of soil.	Moisture added to 50 gm. of soil.	Relative moisture.	Moisture content on the oven-dry basis.	Temperature of incubation.[a]	Presence of protozoa after inoculation (days).							
						1	2	3	4	5	6	7	8
		Gm.		*Per ct.*	°*C.*								
1211	Greenhouse soil.	0	Air-dry.....	0.69	15 to 17	.....	.....	.....	.....	.....	.....	.....	.....
1212	.....do.....	4.96	½ optimum....	9.64	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1213	.....do.....	9.92	1 optimum.....	17.12	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1214	.....do.....	14.95	1½ optimums...	23.54	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1311	Orchard soil...	0	Air-dry.....	.28	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1312	.....do.....	4.47	½ optimum ...	8.46	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1313	.....do.....	8.93	1 optimum.....	15.39	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1314	.....do.....	13.41	1½ optimums...	21.37	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1411	Field soil.....	0	Air-dry.....	.14	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1412	.....do.....	3.48	½ optimum.....	6.63	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1413	.....do.....	6.95	1 optimum.....	12.32	...do...	.....	.....	.....	.....	.....	.....	.....	.....
1414	.....do.....	10.41	1½ optimums...	17.34	...do...	.....	.....	[b] S.C.†	.....	.....	.....	.....	.....

[a] The writer did not think it advisable to include the remainder of Table III representing samples incubated at 5° to 7°, 22° to 24°, 32° to 33°, and at the outdoor temperature, as in no case were any living protozoa found during the period of eight days.

[b] S. C.†=few small ciliates.

Upon examining Table III it is seen that in but one sample of soil (the field soil which had an optimum and a half of moisture) were any active protozoa observed. It was noted that there was a little depression in the sample of soil and a little free available water was present, thus no doubt accounting for the presence of this organism on the third day of incubation, as on no other day and in no other soil were any motile protozoa seen.

In order to be certain that these soils contained cysts of protozoa and to collect some data as to the amount of moisture necessary for excystment and also to note the time of excystment of protozoa when conditions are favorable, to each series of the three different soil samples containing moisture to the amount of half optimum and optimum and a half sterile tap water was added to make the amount two optimums and two and one-half optimums, respectively. These samples were then incubated at the same temperatures as before, and daily examinations for a period of four days were made. (See Table IV.)

TABLE IV.—*Presence of active protozoa in different soils at different temperatures when the moisture conditions were favorable*

Laboratory No.	Kind of soil.	Moisture content.	Relative amount of moisture (in optimums).	Temperature of incubation.	Presence of active protozoa.[a] 8 to 12 hours after inoculation.	30 to 36 hours after inoculation.	Third day after inoculation.	Fourth day after inoculation.
		Per ct.		°C.				
1202	Greenhouse	28.88	2	5 to 7				F†
1204	do	36.66	2½	5 to 7				S.C†F†
1302	Orchard	26.52	2	5 to 7				F†
1304	do	29.05	2½	5 to 7				F†
1402	Field	21.83	2	5 to 7	S.C.†.	S.C.†.	S.C.	
1404	do	25.86	2½	5 to 7	F.†.	F.†.	F.†	
1212	Greenhouse	28.88	2	16 to 17			F.†	
1214	do	33.66	2½	16 to 17			S.C.†† F.††	S.C.†
1312	Orchard	26.52	2	16 to 17		S.C.†.	S.C.† F.†.	S.C.†
1314	do	29.05	2½	16 to 17		F.†.	S.C.†.	F†
1412	Field	21.83	2	16 to 17		S.C.†.	S.C.† F.†.	S.C.† F.††
1414	do	25.86	2½	16 to 17		F.†.	F.††	S.C.†† F†
								F.†
1222	Greenhouse	28.88	2	22 to 24			S.C.†† F.††.	F†
1224	do	33.66	2½	22 to 24		S.C.† F.†.	S.C.†† F.††.	S.C.†F.††
1322	Orchard	26.52	2	22 to 24			S.C.†.	F.††
1324	do	29.05	2½	22 to 24		S.C.†.	S.C.† F.†.	F.††
1422	Field	21.83	2	22 to 24		S.C.†.	S.C.†† F.††.	S.C.†
1424	do	25.86	2½	22 to 24		S.C.† F.†.	S.C.†.	S.C.†
1232	Greenhouse	28.88	2	32 to 33			F.†.	S.C.†
1234	do	33.66	2½	32 to 33		S.C.† F.†.	S.C.†.	S.C.††
1332	Orchard	26.52	2	32 to 33		S.C.†.	S.C.†† L.C.† F.†	F.†
1334	do	29.05	2½	32 to 33	S.C.†.	F.†.	S.C.†† F.†††	F.†
1432	Field	21.83	2	32 to 33	F.†.	F.†.	S.C.††† L.C.† F.†	S.C.† F.†
1434	do	25.86	2½	32 to 33		S.C.† F..	S.C.††.	S.C.†††
1242	Greenhouse	28.88	2	Outdoor temperature.			S.C.†.	S.C.† L.C.†
1244	do	33.66	2½	do			S.C.†.	F.†
1342	Orchard	26.52	2	do		S.C.† F.†.	S.C.†† F.†.	F.†
1344	do	29.05	2½	do		S.C.†.	S.C.† F.††.	S.C.† F.††
1442	Field	21.83	2	do		S.C.† F.†.	S.C.† F.†.	L.C.† F†
1444	do	25.86	2½	do		F.†.	S.C.† F.†.	S.C.† F.††

[a] S.C.=small ciliates, L.C.=large ciliates, F.=flagellates, A.=amebæ, †=few, ††=several, †††=many.

The data presented in Tables III and IV again point to the fact that the supply of sufficient moisture is the limiting factor which influences the presence of protozoa in the active state in the soil, while the temperature, the presence of organic matter, and the soil structure seem to be only secondary factors.

On examining Table IV it becomes apparent that the temperature influences the period of excystment, in that a higher temperature may encourage a more rapid excystment of a greater number of protozoa and that the physical character of the soil may be more or less influential in the movement of the organisms in the soil; yet if the moisture content is not high enough, the protozoa will not be present in the active state.

To find out whether protozoa were always present in the active state in water-logged soils, samples of six soils, three greenhouse and three field soils, which were kept in the laboratory for some time, were put into small bottles, water-logged, and the bottles plugged with rubber stoppers to prevent evaporation, and then allowed to stand in the labo-

ratory. Examinations were made from time to time for a period, and then the samples were placed outside in the open air where the temperature variation was great and examinations were again made. (See Table V.)

TABLE V.—*Presence of active protozoa in water-logged soils, under constant and variable temperatures*

Laboratory No.	Kind of soil.	Presence of protozoa when incubated at room temperature on—[a]		
		May 25.	June 4.	June 7.
1501	Greenhouse	S.C.† L.C.†	S.C.††† L.C.† F.†††	S.C.† L.C.†
1502	do	S.C.††† L.C.†† F.††† A.†	S.C.††† F.††	S.C.††† F.††
1503	do	S.C.††† F.††	S.C.†† F.††	S.C.††† F.†
1504	Field	S.C.††† L.C.† F.††	S.C.†† F.††	S.C.†††. F.†††
1505	do	S.C.††† F.†	S.C.†††	S.C.†††
1506	do	S.C.††† A.†	S.C.††† L.C.† F.†	S.C.†††

Laboratory No.	Kind of soil.	Presence of protozoa when incubated at outdoor temperature on—[a]		
		June 8.	June 16.	June 23.
1501	Greenhouse	S.C.† L. C.†	S.C.† L.C.† F.†	S.C.† F.†
1502	do	S.C.††† F.†	S.C.††† L.C.††	S.C.† F.†
1503	do	S.C.†††	S.C.†††	S.C.†
1504	Field	S.C.††† L.C.† F.†	S.C.††† L.C.††	S.C.† F.†
1505	do	S.C.†††	S.C.†††	S.C.†† F.†
1506	do	S.C.†† L.C.†	S.C.††† F.††	S.C.†† F.†

[a] S. C. = small ciliates; L. C. = large ciliates; F. = flagellates; A. = amebæ; †= few; ††= several; †††= many.

The data given in Table V indicate that living protozoa were always present in all of the water-logged soils during incubation at outside temperature as well as at room temperature. It was noted that the sudden change from the room temperature to the outside temperature did not have any marked effect upon the existence of the organisms in the active condition.

PERIOD OF EXCYSTMENT OF SOIL PROTOZOA

Since active protozoa were not found in normal field soils, the question at once presented itself, How long a period of time was required for soil protozoa to become active in the presence of sufficient moisture, as, for instance, during a heavy fall of rain, and How long will they remain in the active state? In his work with *Colpoda cucullus* Goodey (3) in 1913 found that at 30° C. many were active after an hour. It was suggested by Martin and Lewin (8), as previously noted, that they may become active in a few minutes. To prevent misunderstanding as to the presence of motile protozoa in the soil, the writer in his method of examination proposed a 2-minute examination of each sample—i. e., the soil was in contact with free water no longer than two minutes at each examination. In no case during the entire course of the many examinations of field soils were any protozoa noted to have excysted

during the 2-minute examination, for in no case were any living protozoa found. It was later found with a limited number of soils examined that no protozoa were observed to excyst in a 5- or even 7-minute period. More evidence on this point is being collected.

Some evidence as to the length of time required for the excystment of soil protozoa when sufficient moisture is available is presented in Tables II and IV. As shown in Table IV, at the incubation temperatures of 5° to 7° and 32° to 33° a few small ciliates and flagellates were observed 8 hours after the increased additions of water were made to the soils. It is also seen that in nearly all samples incubated at 15° to 17°, 22° to 24°, 32° to 33°, and at outdoor temperatures some motile protozoa were present after 30 hours. The higher temperatures seemed to be more favorable for the more rapid excystment. This was also found to be true (1) when protozoa were developed in artificial-culture solutions. Small ciliates excysted in as short a period as did the flagellates. In Table II it is shown that after the soils had been in contact with water for 40 hours all of them showed the presence of small ciliates and flagellates. In several samples active large ciliates were also observed.

In order to accumulate more data as to the period of excystment of protozoa a small sample of each of the three soils (samples air-dried and samples containing an optimum amount of moisture and incubated at 22° to 24°, as given in Table III and in the text just following Table III) were added to a few drops of sterile tap water on a glass slide with a large depression in the center. The soil was stirred with a stirring rod and the film spread over the surface of the slide. A careful examination of each sample was made for a period of five minutes, and the slides containing the samples were then placed in the incubator. They were again examined for 5-minute periods at intervals of 15 minutes and 1, 2, 3, 5, 6, and 8 hours. (See Table VI.)

TABLE VI.—*Time required for the excystment of soil protozoa at 22° to 24° C.*

Laboratory No.	First examination.	After 15 minutes.	After 1 hour.	After 2 hours.	After 3 hours.	After 5 hours.	After 6 hours.	After 8 hours.
1221								F.††
1223							F.†	
1321								
1323			S.C.†	S.C.†			S.C.†	
1421								
1423								

[a] S. C.†= few small ciliates; F.†=few flagellates; F.††=several flagellates.

From the data recorded in Table VI it will be noted that at 22° to 24° protozoa (small ciliates) may excyst within two hours after the protective cysts come in contact with available moisture. Flagellates and other

small ciliates are seen to excyst in from six to eight hours after the immersion of the cysts in water. From the limited amount of study given to this point no conclusive statement as to the relative length of time required for the excystment of soil protozoa can be made. Nevertheless, the writer is of the opinion that under normal conditions protozoa excyst seldom, if at all, in as little as two minutes. There may be cases, however, as where the protective cyst is partially ruptured either by mechanical means or otherwise or where the moisture conditions are almost favorable enough for excystment, in which the organisms will become active in less than two minutes; but under ordinary normal conditions it seems doubtful from the examinations already made whether they can become active in this period of time at 22° to 24°. The indications (Table IV) are that excystment goes on more rapidly at higher temperatures. In all probability the original moisture content of the soil plays a part in determining the length of time which must elapse before the organisms become active. Likewise, different types of protozoa will prefer different conditions (1) and may excyst sooner at one temperature than at another. Further study on this point will be made.

SUMMARY

Under the conditions recorded in this paper the following observations as to the activity of soil protozoa seem to be justified:

(1) Under ordinary greenhouse conditions small ciliates, flagellates, and amebæ are active in some soils, but their presence is very limited.

(2) Active protozoa (small ciliates, large ciliates, flagellates, and amebæ) do not seem to be present in field soils with a normal moisture content and even when the moisture content is slightly supernormal, and, hence, they would not be a limiting factor in the soil.

(3) All field soils contain cysts of protozoa the organisms of which become active when conditions become favorable.

(4) The moisture content of the soil is the primary influencing factor which determines the presence or absence of the active protozoa in the soil, while the temperature, the presence of organic matter, and the physical properties of the soil are secondary factors.

(5) Soon after standing water is accumulated, as after a heavy rain, some protozoa will excyst and be active as long as the moisture content is favorable. Active protozoa seem to be always present in free standing soil water.

(6) Active protozoa are present in water-logged soils at constant and variable temperatures.

(7) Under normal conditions it would seem that protozoa can not excyst in 2 minutes. Small ciliates can excyst in 1 to 2 hours at 22° to 4° C.; at the same temperature flagellates can excyst in 6 to 8 hours and large ciliates can excyst in 40 hours.

LITERATURE CITED

(1) CUNNINGHAM, Andrew.
1915. Studies on soil protozoa. *In* Jour. Agr. Sci., v. 7, pt. 1, p. 49-74.

(2) GOODEY, T.
1911. A contribution to our knowledge of the protozoa of the soil. *In* Proc. Roy. Soc. [London], s. B, v. 84, no. 570, p. 165-180, 1 fig., pl. 4.

(3) ———
1913. The excystation of Colpoda cucullus from its resting cysts, and the nature and properties of the cyst membranes. *In* Proc. Roy. Soc. [London], s. B, v. 86, no. 589, p. 427-439, 2 fig.

(4) ———
1914. Investigations on the protozoa of soil. (Abstract.) *In* Rpt. 83d Meeting Brit. Assoc. Adv. Sci., 1913, p. 775.

(5) KOCH, G. P.
1915. Soil protozoa. *In* Jour. Agr. Research, v. 4, no. 6, p. 511-559. Literature cited, p. 558-559.

(6) MARTIN, C. H.
1913. The presence of protozoa in soils. *In* Nature, v. 91, no. 2266, p. 111.

(7) ——— and LEWIN, K. R.
1914. Some notes on soil protozoa. *In* Phil. Trans. Roy. Soc. [London], s. B, v. 205, p. 77-94, pl. 5-6.

(8) ——— ———
1915. Notes on some methods for the examination of soil protozoa. *In* Jour. Agr. Sci., v. 7, pt. 1, p. 106-119, pl. 2-3.

(9) RUSSELL, E. J., and GOLDING, J.
1911. Sewage sickness in soil, and its amelioration by partial sterilization. *In* Jour. Soc. Chem. Indus., v. 30, no. 8, p. 470-474.

(10) ——— ———
1912. Investigations on "sickness" in soil. *In* Jour. Agr. Sci., v. 5, pt. 1, p. 27-47.

(11) ——— and PETHERBRIDGE, F. R.
1912. Investigations on "sickness" in soil. II. "Sickness" in glasshouse soils. *In* Jour. Agr. Sci., v. 5, pt. 1, p. 86-111, pl. 2-5.

(12) SHERMAN, J. M.
1914. The number and growth of protozoa in soil. *In* Centbl. Bakt. [etc.] Abt. 2, Bd. 41, No. 18/23, p. 625-630.

(13) WOLFF, Max.
1909. Der Einfluss der Bewässerung auf die Fauna der Ackerkrume mit besonderer Berücksichtigung der Bodenprotozoen. *In* Mitt. Kaiser Wilhelms Inst. Landw. Bromberg, Bd. 1, Heft 4, p. 382-401, 49 fig.

BERIBERI AND COTTONSEED POISONING IN PIGS[1]

[PRELIMINARY NOTE]

By GEORGE M. ROMMEL, *Chief, Animal Husbandry Division, Bureau of Animal Industry*, and EDWARD B. VEDDER, *Captain, Medical Corps, United States Army*

SO-CALLED COTTONSEED POISONING OF ANIMALS

Cottonseed meal is one of the most valuable feedstuffs at the command of the American stockman. After the animal has digested it, the value of the residue as fertilizer is about three-fourths the original value of the meal. The United States uses only part of the cottonseed meal which it produces, and one of the reasons which prevent a larger domestic consumption of this by-product of the cotton industry is the danger that sickness and death may follow its use.

Cattle fed for more than 90 to 120 days on a heavy cottonseed-meal ration (6 pounds or more per head daily) become lame, and their eyes discharge freely, blindness often resulting. Deaths may occur, especially in young animals. Pigs are peculiarly susceptible to the effects of cottonseed meal, possibly because they are usually fed a larger quantity of the meal in proportion to their body weight. In feeding pigs, symptoms of sickness may appear at any time after three weeks of feeding, and deaths frequently occur with little warning.

Various systems of feeding cottonseed meal to pigs have been devised. Some of them appear to minimize its danger somewhat, but none of them prevent it entirely. This product, therefore, can not be regarded as a safe feed for pigs in the combinations in which it has heretofore usually been fed.

Among the more pronounced symptoms observed in pigs suffering from the effects of cottonseed-meal feeding are diarrhea; a harsh, rough, curly coat; paralysis; and shortness of breath. Emaciation and dropsical conditions are frequently observed. The disease manifests two forms—acute or chronic.

The acute form is much more serious to the farmer, because pigs are attacked by it with little warning and may be dead before any indications of disease are noticed. The largest and best nourished pigs are often the ones attacked. The attack is sudden and sharp. The pig experiences extreme shortness of breath and suffers the most intense pain. If he recovers, recurrences of the attack are likely, especially if the pig is a heavy feeder. Subsequent attacks may end fatally, or the disease may assume the chronic form.

[1] This opportunity is taken to express appreciation of the cooperation of Dr. Adolph Eichhorn, Chief of the Pathological Division of the Bureau of Animal Industry, in having made the necessary post-mortem examinations of pigs used in these experiments.

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In the chronic form fatal results may not occur for a considerable time. The symptoms persist if the feed is not changed, and the pig appears to develop a certain degree of immunity to the effects of the disease. His condition, however, is continually, although slowly, declining. Pigs suffering from this form of the disease may live for a year or more on a cottonseed-meal ration.

On post-mortem examination, pigs which have died from the effects of cottonseed-meal feeding show large quantities of fluid in the abdominal and thoracic cavities and in the pericardial sac. The kidneys, liver, spleen, and small intestines are usually congested. In some cases the membrane lining the stomach is eroded. The lungs are very edematous, especially in pigs which have died from sudden acute attacks. The heart is enlarged.

SIMILARITY OF SYMPTOMS OF COTTONSEED POISONING AND OF BERIBERI

These conditions bear a striking resemblance to those seen in the disease known as beriberi in man, which, according to Vedder,[1] results "from faulty metabolism * * * and is directly caused by the deficiency of certain vitamines in the food."

Beriberi in human beings is usually caused by a diet of highly milled rice and is never known to result from a diet of rice from which the pericarp and aleurone layer of the grain have not been removed. However, the disease may be caused by diets of which rice forms no part whatever. For example, a diet of bread or macaroni alone made from highly milled wheat flour will produce beriberi. Birds (chickens and pigeons) are generally used in the laboratory study of beriberi because they readily develop the chronic or "dry" form when fed on a diet of highly milled rice for a sufficient time, but they will also develop the disease if fed on an exclusive diet of white wheat bread.

Beriberi in pigs is not frequently reported in the literature on the subject. Braddon[2] reports, without details, the case of a pig fed on polished rice. The pig developed paralysis in about a month and died suddenly. It is believed that until this year this was the only case of the kind recorded.

EXPERIMENTS TO COMPARE EFFECTS OF FEEDING POLISHED RICE AND COTTONSEED MEAL

On August 31, 1915, the writers began a series of experiments to determine (*a*) whether the "wet" or acute form of beriberi could be produced in pigs on a diet of polished rice, and (*b*) whether the disease heretofore called "cottonseed poisoning" in pigs is not really beriberi.[3] Four pigs

[1] Vedder, E. B. Beriberi. p. viii. New York, 1913.

[2] Braddon, W. L. The Cause and Prevention of Beri-Beri. p. 355. London, New York, 1907.

[3] It should be noted that Withers and Carruth made no extensive use of pigs in their investigations on gossypol. (Withers, W. A., and Carruth, F. E. Gossypol, the toxic substance in cottonseed meal. In Jour. Agr. Research, v. 5, no. 7, p. 261–288, pl. 25–26. 1915.)

were fed a ration of 9 parts (by weight) of steamed polished rice and 1 part of tankage, and four a ration of 2 parts of corn meal and 1 part of cottonseed meal. On October 24 the ration of the latter pigs was changed to equal parts by weight of corn meal and cottonseed meal. None of these pigs had received rice or cottonseed meal before they entered the experiment.

On September 8 one of the pigs on rice began to breathe with difficulty. On the 10th this condition was pronounced, and he refused to eat. On September 14 these symptoms rapidly became more severe, paralysis developed, and the pig died shortly before noon. The ante-mortem symptoms were what one would expect to see in an acute case of so-called cottonseed poisoning. They were, in fact, the symptoms of wet beriberi. The post-mortem examination showed serous fluid in the pericardial sac and in the thoracic and abdominal cavities. The heart was enlarged and the cardiac muscle congested. The lungs were decidedly edematous and mottled with a fair number of small subpleural hemorrhages. The liver was intensely congested and enlarged. The spleen was apparently unaltered, but was dark in color. The stomach showed several erosions in the mucosa, and the walls were thickened. The small intestines were slightly congested. Many of the mesenteric glands were enlarged and congested. Both kidneys were congested, especially at the apices, which were deep cherry-red in color. The bladder was distended with urine, which contained a large amount of albumin. Except for the large quantity of albumin, this is exactly what one would expect to find in a beriberi necropsy. It is also what is found in an acute cottonseed-meal necropsy.

On September 21 four additional pigs were placed on the same steamed rice and tankage ration (9:1). On September 29 one of these pigs became sick and on September 30 it refused to eat. He recovered and regained his normal appetite, but died on October 29, after having been on the rice diet for 38 days. The ante-mortem symptoms corresponded closely to those of the first pig to die, but the post-mortem examination did not give such clear-cut results. The sciatic nerves of this pig were dissected out immediately after the post-mortem examination and, after being treated by the Marchi method, showed considerable degeneration of the nerve fibers.

The writers believe that pigs fed a ration in which rice is the chief component will develop beriberi as do human beings, but much more quickly. Weight is given to this belief by the experience of Moore,[1] who lost pigs fed on "rice meal" [2] from a disease which Hadwen [3] suspects to be beriberi.

[1] Moore, P. H. Hog-feeding experiments. In Canada Exp. Farms Rpts. [1912]/13, p. 611–613. 1914.
——— Preliminary note on the effects of feeding rice meal to pigs. In Canada Dept. Agr. Rpt. Vet. Dir. Gen. [1913]/14, p. 137–141. 1915.
[2] Apparently not the rice meal of our Southern States.
[3] Hadwen, S. Notes on the pathology and symptoms of rice-meal fed pigs. In Canada Dept. Agr. Rpt. Vet. Dir. Gen. [1913]/14, p. 140. 1915.

The remaining 10 pigs are being continued on the rice and cottonseed-meal rations. At the time this article is written they have been almost 90 days on these feeds. All the pigs are sick, and the same symptoms have appeared in each lot. In fact, it may be said that the most typical and acute cottonseed-meal symptoms are seen among the pigs receiving rice.

A mature brood sow, weighing 400 pounds, due to farrow on November 14, 1915, was placed on a cottonseed-meal ration on September 2. She was started on a ration of 4 parts of corn meal and 1 part of cottonseed meal, the quantity of corn meal being gradually decreased until, on October 1, she was receiving equal parts of corn meal and cottonseed meal. Up to November 14 she had eaten 134.65 pounds of cottonseed meal. She showed no serious sign of sickness, except nausea on November 4, when she vomited. At 8 p. m. on November 13 she began to farrow and delivered 9 pigs, the last one being born at 4 o'clock the following morning. Four of these pigs were born dead, and of those born alive all but one died in a few minutes. The last pig born lived less than eight hours.

Post-mortem examinations were made of seven of these pigs, four of which had been born alive. All of them showed enlarged hearts, and serum was found in the pericardial sac, the thoracic cavity, and the abdominal cavity. The quantity of serum was a little greater in the pigs born alive than in those born dead. In the pigs born alive there was some injection in the lungs, liver, and small intestines, but none in those born dead. There were no alterations in the kidneys of any of the pigs born alive or dead.

These pigs were very well developed, plump, and apparently had been well nourished. They averaged slightly over 2 pounds 6 ounces in weight. The analogy with infantile beriberi is apparent. Yet the dam had never eaten rice, and the only assignable cause for the death of her litter was the cottonseed meal in her ration. Her breeding record for previous farrowings is as follows:

Item.	1914	1915
Date of farrowing	Apr. 7	Apr. 2
Number of pigs	5	12
Number born alive	5	9
Number raised	4	5

The sow was a good breeder, and difficult labor can not be given as the cause of the death of the litter.

CONCLUSIONS

The studies of the writers seem to lead to three general conclusions:

(1) Pigs are susceptible to beriberi when fed on vitamine-deficient rations, such as rice. The disease develops much more rapidly in pigs

than in man. In man symptoms rarely, if ever, appear before 90 days. In pigs the writers have found symptoms of a pronounced character in from 8 to 10 days.

(2) It is believed that the so-called cottonseed poisoning of pigs is a deficiency disease, analogous to the disease known as beriberi in man, if not indeed identical with it. Acute cottonseed poisoning corresponds to wet beriberi, and the chronic form to dry beriberi.

(3) The cause of the so-called cottonseed poisoning is probably a deficiency in the ration, causing, among other manifestations, profound changes in the nervous system.

At first thought this theory is not justified. Beriberi results from a ration of highly milled rice, because substances vitally necessary to the animal organism have been removed from the rice grain in the process of milling. When pigs suffer from so-called cottonseed poisoning, it is only when cottonseed meal has been added to the ration. Pigs are seldom, if ever, fed on cottonseed meal alone.

The following explanation of this condition is offered: The grain with which the cottonseed meal is most frequently combined is corn. Corn is notoriously deficient as a single feed for animals, and it must be properly balanced to be fed satisfactorily. The excellent results in feeding pigs which can be obtained from rations of corn meal and skim milk or other animal products, such as tankage, blood meal, fish meal, etc., are out of all proportion to the facts indicated by the conventional chemical analyses of protein, carbohydrates, and fat. When corn meal is fed with cottonseed meal, a combination is made of two feeds both of which are deficient.

The writers are engaged in further studies of this subject to determine more exactly the effects of cottonseed meal when fed in the ration of the pig, and to determine whether methods similar to those used to prevent beriberi in man can be practically applied to prevent the so-called cottonseed poisoning of pigs.